

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 99-372-F1)

In re Application of:	Welcher et al.)	
Serial No.:	11/200,389)	Before the Examiner: J. Seharaseyon
Filed:	August 8, 2005)	Group Art Unit: 1647
For:	Interferon-like Molecules and Uses Thereof)	Confirmation No.: 3469

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

RESPONSE TO OFFICE ACTION MAILED NOVEMBER 23, 2007

Responsive to the Office Action mailed November 23, 2007, Applicants respectfully request reconsideration of the above-identified application in view of the following amendments and remarks.

Amendments to the Specification: Pursuant to 37 C.F.R. § 1.121, Applicants present the amendments to the specification at page 2, marked up to show changes made relative to the immediate prior version of the specification.

Amendments to the Claims: Pursuant to 37 C.F.R. § 1.121, Applicants present a complete listing of the claims, including marked up versions of all currently amended claims, at pages 3-5

Remarks: Applicants' Remarks begin on page 6 of this paper.

Amendments to the Specification under 37 C.F.R. § 1.121

Please amend the specification at page 1, line 1 as follows:

INTERFERON-LIKE MOLECULES PROTEINS AND USES THEREOF

Amendments to the Claims under 37 C.F.R. § 1.121

Claim 1 (original): An isolated polypeptide comprising an amino acid sequence:

- (a) as set forth in SEQ ID NO: 5; or
- (b) encoded by the DNA insert in ATCC Deposit No. PTA-976.

Claim 2 (currently amended): An isolated polypeptide comprising:

- (a) ~~an~~ the amino acid sequence as set forth in SEQ ID NO: 6, optionally further comprising an amino-terminal methionine;
- (b) ~~an amino acid sequence that is at least about 70 percent identical to the amino acid sequence set forth in SEQ ID NO: 5, wherein the polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation; or~~
- (c) ~~a fragment of the amino acid sequence set forth in SEQ ID NO: 5 comprising at least about 25 amino acid residues, wherein the polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation, or is antigenic.~~

Claim 3 (cancelled).

Claim 4 (currently amended): An isolated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence:

- (a) as set forth in SEQ ID NO: 4;
 - (b) of the DNA insert in ATCC Deposit No. PTA-976; ~~or~~
 - (c) encoding a polypeptide as set forth in SEQ ID NO: 5; ~~or~~
 - (d) ~~that hybridizes to the complement of the nucleotide sequence of any of (a)-(c) under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences;~~
- wherein the encoded polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation.

Claims 5-7 (cancelled).

Claim 8 (currently amended): A composition comprising the polypeptide of any of Claims 1, 2, or 3 4, and a pharmaceutically acceptable formulation agent.

Claim 9 (original): The composition of Claim 8, wherein the pharmaceutically acceptable formulation agent is a carrier, adjuvant, solubilizer, stabilizer, or anti-oxidant.

Claim 10 (currently amended): The composition of Claim 8, wherein the polypeptide comprises ~~an~~ the amino acid sequence as set forth in SEQ ID NO: 6.

Claim 11 (currently amended): A polypeptide comprising a derivative of the polypeptide of any of Claims 1, 2, or 3 4.

Claim 12 (original): The polypeptide of Claim 11 that is covalently modified with a water-soluble polymer.

Claim 13 (original): The polypeptide of Claim 12, wherein the water-soluble polymer is polyethylene glycol, monomethoxy-polyethylene glycol, dextran, cellulose, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide copolymers, polyoxyethylated polyols, or polyvinyl alcohol.

Claim 14 (currently amended): A fusion polypeptide comprising the polypeptide of any of Claims 1, 2, or 3 4 fused to a heterologous amino acid sequence.

Claim 15 (original): The fusion polypeptide of Claim 14, wherein the heterologous amino acid sequence is an IgG constant domain or fragment thereof.

Claim 16 (currently amended): A polypeptide produced by a process comprising culturing a host cell comprising a vector comprising a nucleic acid molecule comprising a nucleotide sequence:

(a) as set forth in SEQ ID NO: 4;
(b) of the DNA insert in ATCC Deposit No. PTA-976; or
(c) encoding a polypeptide as set forth in SEQ ID NO: 5; or
(d) ~~that hybridizes to the complement of the nucleotide sequence of any of (a)-(c)~~
under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences,
wherein ~~the polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation;~~
under suitable conditions to express the polypeptide, and optionally isolating the polypeptide from the culture.

Claims 17-18 (cancelled).

Claim 19 (currently amended): The polypeptide of ~~any of Claim~~[[s]] 16, 17, ~~or 18~~, wherein the host cell is a eukaryotic cell.

Claim 20 (currently amended): The polypeptide of ~~any of Claim~~[[s]] 16, 17, ~~or 18~~, wherein the host cell is a prokaryotic cell.

REMARKS

Claims 2, 4, 8, 10, 11, 14, 16, 19, and 20, as amended, and claims 1, 9, 12, 13, and 15 are pending in the instant application. Claims 3, 5-7, 17, and 18 have been canceled without prejudice or disclaimer. No new matter has been added as a result of the above-described amendments. The rejections set forth in the Office Action have been overcome by amendment or are traversed by argument below.

1. Claim of priority

The Office Action states that the instant application appears to claim subject matter disclosed in U.S. Application No. 09/927,850 (the '850 application), and that Applicants must amend the specification to insert a reference to the '850 application as the first sentence of the specification if Applicants intend to rely on the filing date of the '850 application under 35 U.S.C. §§ 119(c), 120, 121, or 365(c).

Coinciding with the submission of this Response, Applicants have submitted a Petition for an Unintentionally Delayed Domestic Priority Claim in order to amend the first sentence of the instant application to insert a reference to the '850 application.

2. Objections to the Specification

The Office Action contains an objection to the specification because the title of the invention is not descriptive. The Action states that a new title is required that is clearly indicative of the invention to which the claims are directed.

Applicants have amended the title to read: "Interferon-Like Proteins and Uses Thereof," which Applicants contend is clearly indicative of the invention to which the claims are directed. Applicants, therefore, respectfully request that this ground of objection be withdrawn.

The Office Action asserts an objection to the specification because of the improper use of the trademark "Quick Spin" and "Taq polymerase," which the Action states should be capitalized and accompanied by generic terminology.

Applicants have searched the U.S. Patent and Trademark Office Trademark Electronic Search System (TESS), but have not found a record for the term "Quick Spin" with respect to

Qiagen's G-50 column. Applicants were also unable to find a record for the term "Taq polymerase." Applicants contend that because the terms "Quick Spin" and "Taq polymerase" do not appear to be registered trademarks, these terms do not to be capitalized or accompanied by generic terminology. Applicants respectfully request that this ground of objection be withdrawn.

3. Rejection of claims 5, 6, and 16-20 under 35 U.S.C. § 112, second paragraph

Claims 5, 6, and 16-20 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that applicants regard as the invention.

a. Hybridization conditions

Claims 5, 6, and 16-18 are rejected for reciting the term "hybridization conditions," which the Action states is insufficiently defined in the specification.

Applicants have cancelled claims 5, 6, 17, and 18, and have amended claim 16 to delete subpart (d). As claim 16 no longer recites hybridization conditions, Applicants respectfully request that this ground of rejection be withdrawn.

b. Polypeptide production

Claims 16-18 are rejected for being indefinite since it is unclear how the polynucleotide complements of claims 16(d), 17(c), and 18(b) can encode a polypeptide having the activity of the polypeptides disclosed in the specification.

Applicants have cancelled claims 17 and 18, and have amended claim 16 to delete subpart (d). As claim 16 no longer recites subpart (d), Applicants respectfully request that this ground of rejection be withdrawn.

c. IFN-L polypeptide

Claims 2, 5, and 17 are rejected as being vague and indefinite for the reciting the term "at least about."

Applicants have cancelled claims 5 and 17, and have amended claim 2 to delete subpart (b). As claim 2 no longer recites the term "about," Applicants respectfully request that this ground of rejection be withdrawn.

Applicants submit that the claims as amended are in condition for allowance and respectfully request that the rejections under 35 U.S.C. § 112, second paragraph, be withdrawn.

4. Rejection of claims 1-20 under 35 U.S.C. § 112, first paragraph

a. Rejection of claims 1-20 under the enablement requirement of 35 U.S.C. § 112, first paragraph

Claims 1-20 are rejected under 35 U.S.C. § 112, first paragraph, as not being enabled by the specification such that one of skill in the art could make and use the invention as claimed. The Action specifically asserts that all possible variants of SEQ ID NO: 5 are not enabled, and that the amount of experimentation required to determine these variants would be undue.

Applicants respectfully disagree with the Action's assertion that the claimed variants of SEQ ID NO: 5 are not enabled. Nevertheless, solely in an effort to expedite prosecution of the pending claims to allowance, Applicants have cancelled claims 5, 6, 17, and 18, and have deleted claims 2(b), 2(c), 4(d), and 16(d). As the pending claims no longer recite variants of SEQ ID NO: 5, Applicants respectfully request that this ground of rejection be withdrawn.

Applicants reserve the right to pursue claims directed to the cancelled or deleted subject matter in a timely filed continuation or divisional application, or alternatively, reintroduce the cancelled or deleted subject matter in the instant application at such time as the Office indicates that the pending claims are otherwise in condition for allowance.

The Action also asserts that phrases such as "an amino acid sequence" and "a nucleic acid molecule" in the claims read upon various variants and fragments. Applicants have cancelled claim 6 and 18, and have amended claims 2(a) and 10 to recite "the" rather than "a" or "an." Applicants, therefore, respectfully request that this ground of rejection be withdrawn.

The Action further asserts that Applicants' referral to the deposit of PTA-976 in the specification and in claims 1, 4, 5, 16 and 17 is an insufficient assurance that all of the conditions of 37 C.F.R. §§ 1.801-1.809 have been met. The Action states that Applicants must submit a statement

by an attorney of record over his or her signature, stating that a deposit has been made under the terms of the Budapest Treaty and that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent. The Action further states that the instant specification must be amended to recite the date of the deposit and the complete name and address of the depository, and that the claims must be amended to recite the accession number.

Pursuant to the Examiner's request, Applicants' representative submits the following statement: Applicants deposited cDNA encoding human IFN-L polypeptide with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209. The deposit was accepted by the ATCC, an International Depository Authority, under the provisions of the Budapest Treaty, and the deposit was designated as PTA-976. A copy of the ATCC receipt for this deposit, showing the patent deposit designation (Accession No. PTA-976) and the date on which the deposit was received by the ATCC (November 23, 1999), is attached. Pursuant to 37 C.F.R. § 1.808(a)(2), the deposit was made under conditions that assure that all restrictions imposed by the depositors on the availability to the public of the deposited material would be irrevocably removed upon the granting of a patent relying on the deposited biological material. In making the deposit, Applicants acknowledged their responsibility, pursuant to 37 C.F.R. § 1.805, to provide a replacement or supplemental deposit if the depository possessing the deposit is unable to furnish samples thereof or is able to furnish samples thereof but the deposit has become contaminated or has lost its capability to function as described in the specification. With regard to the assertion that the date of the deposit and the complete name and address of the depository is not referred to in the body of the specification, Applicants respectfully direct the Examiner's attention to page 92, lines 25-28 of the specification as-filed, where Applicants disclose that a deposit of cDNA encoding human IFN-L polypeptide, subcloned into pSPORT1 (Gibco BRL) and transfected into *E. coli* strain DH10B, having Accession No. PTA-976, were made with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on November 23, 1999. With regard to the assertion that the accession number of the deposit is not referred to in the claims, Applicants respectfully direct the Examiner's attention to claims 1(b), 4(b), and 16(b), as originally filed. Applicants contend that all the requirements of 37 C.F.R. §§ 1.801-1.809 have been met. *In re*

Lundak, 225 U.S.P.Q. 90 (Fed. Cir. 1985). Withdrawal of this rejection is therefore respectfully solicited.

Applicants submit that the claims as amended are in condition for allowance, and respectfully request that the rejections under the enablement requirement of 35 U.S.C. § 112, first paragraph, be withdrawn.

b. Rejection of claims 1-20 under the written description requirement of 35 U.S.C. § 112, first paragraph

Claims 1-20 have been rejected as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Action asserts that the specification does not disclose all possible variants of nucleic acid molecules that hybridize to the complement of the claimed nucleotide sequences with more than 21% mismatch. The Action also asserts that specification has failed to disclose any other sequence contemplated in the instant claims including IFN-L fragments and other variants, and thus the skilled artisan cannot envision the detailed chemical structures of the claimed polypeptide sequences. Finally, the Action asserts that the species specifically disclosed are not representative of the genus because the genus is highly variant.

Applicants respectfully disagree with the Action's assertion that the specification does not contain an adequate written description of the claimed invention. Nevertheless, solely in an effort to expedite prosecution of the pending claims to allowance, Applicants have cancelled claims 5, 6, 17, and 18, and have deleted claims 2(b), 2(c), 4(d), and 16(d). As the pending claims no longer recite the variants described above, Applicants respectfully request that this ground of rejection be withdrawn.

Applicants reserve the right to pursue claims directed to the cancelled or deleted subject matter in a timely filed continuation or divisional application, or alternatively, reintroduce the cancelled or deleted subject matter in the instant application at such time as the Office indicates that the pending claims are otherwise in condition for allowance.

Applicants submit that the claims as amended are in condition for allowance, and respectfully request that the rejection under the written description requirement of 35 U.S.C. § 112, first paragraph, be withdrawn.

5. Rejection of claims 1-20 under 35 U.S.C. § 102(e)

The Office Action asserts a rejection of claims 1-20 under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,433,145 (the '145 patent).

Pursuant to 37 C.F.R. § 41.202, Applicants suggest that an interference be declared between the instant application and U.S. Patent No. 6,433,145 (the '145 patent). The '145 patent issued on August 13, 2002 from U.S. Application No. 09/487,792 (the '792 application), which was filed on January 20, 2000. The '792 application is a continuation-in-part of U.S. Application No. 09/358,587 and International Application No. PCT/US99/16424, both filed on July 21, 1999, and claims the benefit of U.S. Provisional Application No. 60/093,643, filed July 21, 1998.

Applicants believe claims 1 and 51 of the '145 patent interfere with claims 1 and 2 of the instant application. Claims 1 and 51 of the '145 patent read as follows:

1. An isolated protein comprising a polypeptide having an amino acid sequence selected from the group consisting of:

- (a) amino acids 1 to 207 of SEQ ID NO:2;
- (b) amino acids 7 to 207 of SEQ ID NO:2;
- (c) amino acids 2 to 207 of SEQ ID NO:2; and
- (d) amino acids 28 to 207 of SEQ ID NO:2.

51. An isolated protein comprising a polypeptide having an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment has anti-viral activity;
- (b) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment inhibits bone marrow proliferation;
- (c) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment activates the Jak/Stat pathway; and
- (d) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.

Claims 1 and 2 of the instant application read as follows:

1. An isolated polypeptide comprising an amino acid sequence:
 - (a) as set forth in SEQ ID NO: 5; or
 - (b) encoded by the DNA insert in ATCC Deposit No. PTA-976.
2. An isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 6, optionally further comprising an amino-terminal methionine.

Applicants propose the following counts:

1. An isolated polypeptide comprising the amino acid sequence of amino acids 1 to 207 of SEQ ID NO:2 of U.S. Patent No. 6,433,145.
2. An isolated polypeptide comprising the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment has anti-viral activity, inhibits bone marrow proliferation, activates the Jak/Stat pathway, or binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.

A comparison between the proposed counts, claims 1(a) and 51 of the '145 patent, and claims 1(a) and 2 of the instant application is provided in the claim chart below:

Count 1	Claim 1(a) of '145 patent	Claim 1(a) of the instant application
An isolated polypeptide	An isolated protein comprising a polypeptide	An isolated polypeptide
comprising the amino acid sequence of amino acids 1 to 207 of SEQ ID NO:2. of U.S. Patent No. 6,433,145.	having an amino acid sequence [that is] amino acids 1 to 207 of SEQ ID NO:2[.]	comprising an amino acid sequence . . . as set forth in SEQ ID NO: 5
Count 2	Claim 51 of '145 patent	Claim 2 of the instant application
An isolated polypeptide	An isolated protein comprising a polypeptide	An isolated polypeptide
comprising the amino acid	having an amino acid	comprising the amino acid

sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2,	sequence [that is] the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2,	sequence as set forth in SEQ ID NO: 6
wherein the fragment has anti-viral activity, inhibits bone marrow proliferation, activates the Jak/Stat pathway, or binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.	wherein the fragment has anti-viral activity; . . . wherein the fragment inhibits bone marrow proliferation; . . . wherein the fragment activates the Jak/Stat pathway; [or] wherein the fragment binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.	[While claim 2 does not expressly recite this limitation, the polypeptide recited in the claim, which is the mature form of the full-length IFN-L polypeptide, would inherently have such properties.]

The sequence alignment provided in Exhibit A indicates that the amino acid sequence of SEQ ID NO: 5 of the instant application shares 100% sequence identity with amino acids 1 to 207 of SEQ ID NO: 2 of the '145 patent. Applicants contend that because the amino acid sequence of SEQ ID NO: 5 of claim 1(a) of the instant application and amino acids 1 to 207 of SEQ ID NO: 2 of claim 1(a) of the '145 patent share 100% identity, the subject matter of either claim would, if prior art, have anticipated the subject matter of the other claim.

The sequence alignment provided in Exhibit B indicates that the amino acid sequence of SEQ ID NO: 6 of the instant application shares 100% sequence identity with amino acids 30 to 207 of SEQ ID NO: 2 of the '145 patent. The amino acid sequence of SEQ ID NO: 6 is, therefore, a fragment of amino acid residues 1 to 207 of SEQ ID NO: 2 of the '145 patent. Applicants contend that because the amino acid sequence of SEQ ID NO: 6 is the mature form of the amino acid sequence of SEQ ID NO: 2 of the '145 patent (as well as of the amino acid sequence of SEQ ID NO: 5 of the instant application), the amino acid sequence of SEQ ID NO: 6 would have anti-viral

activity, inhibit bone marrow proliferation, activate the Jak/Stat pathway, or bind an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO: 2. Applicants also contend that because the amino acid sequence of SEQ ID NO: 6 of claim 2 of the instant application shares 100% identity with a fragment of the amino sequence of SEQ ID NO: 2 of the '145 patent, and the mature form would have anti-viral activity, inhibit bone marrow proliferation, activate the Jak/Stat pathway, or bind an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO: 2, the subject matter of either claim 51 of the '145 patent and claim 2 of the instant application would, if prior art, have anticipated the subject matter of the other claim.

Applicants contend that the Declaration Pursuant to 37 C.F.R. § 1.131 which is provided in Exhibit C, and which was submitted on June 24, 2004 for U.S. Application No. 09/927,850 (from which the instant application claims the benefit of priority as a continuation application), establishes that Applicants will prevail on priority were an interference between the instant application and the '145 patent to be declared. In particular, the Declaration provides copies of forty-one (41) pages from the inventors' laboratory notebook showing conception of the claimed invention before July 21, 1998. The laboratory notebook pages show that a genomic cloning approach was used to identify the nucleic acid sequence of human interferon-like polypeptide (*see* page 34 of laboratory notebook pages). Specifically, three genomic clones were identified as containing nucleic acid sequences encoding at least a portion of human interferon-like polypeptide (clones 2, 6, and 7; *see* page 40 of laboratory notebook pages). The nucleic acid sequences from these clones were isolated and then re-cloned into a suitable sequencing vector. One of the three genomic clones was determined to contain a partial nucleic acid sequence for human interferon-like polypeptide and another genomic clone (clone 6) was determined to contain a full-length nucleic acid sequence for human interferon-like polypeptide (*see* page 62 of laboratory notebook pages). The amino acid sequence of human interferon-like polypeptide was determined from the latter nucleic acid sequence.

The Declaration also provides copies of ten (10) pages from a Research Summary prepared by the inventors showing that the invention disclosed and claimed in the instant patent application was diligently reduced to practice. Specifically, the Research Summary shows that the inventors performed experiments in order to determine the function of protein encoded by the nucleic acid sequence described above, and that once the function of the protein had been determined, the inventors prepared a Research Summary and submitted that Summary to the legal department of

Amgen Inc., the assignee of the instant application. More particularly, the Research Summary shows that several versions of the human and rat IFN-L proteins were produced in a mammalian expression system (*see* page 7 of Research Summary) and that rat IFN-L:Fc fusion protein treatment of several cell lines was found to cause phosphorylation of cellular proteins (*see* page 10 of Research Summary). Thus, the Declaration and attachments provided in Exhibit C establish that Applicants will prevail on priority were an interference between the instant application and the '145 patent to be declared.

Applicants note that claims 1(a) and 2 of the instant application were not added or amended in order to provoke an interference. Applicants contend, therefore, that pursuant to 37 C.F.R. § 41.202(a)(5), a claim chart showing the written description for claims 1(a) and 2 in the specification need not be provided.

Finally, Applicants submit the following chart showing where the instant disclosure provides a constructive to reduction of practice within the scope of the interfering subject matter:

Count 1	Reduction to Practice in Instant Disclosure
An isolated polypeptide comprising the amino acid sequence of amino acids 1 to 207 of SEQ ID NO:2. of U.S. Patent No. 6,433,145.	page 4, lines 9-12; page 7, lines 28-30; page 11, lines 4-5; page 95, lines 10-16; and Figures 2A-2B.
Count 2	Reduction to Practice in Instant Disclosure
An isolated polypeptide comprising the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment has anti-viral activity.	page 4, lines 16-19; page 12, line 28 to page 13, line 3; page 7, lines 28-30; page 95, lines 10-16; page 103, lines 20-27; and Figures 2A-2B.

In view of the above discussion, Applicants respectfully suggest that an interference be declared between the instant application and the '145 patent.

CONCLUSIONS

Applicants respectfully contend that all conditions of patentability are met in the pending claims as amended. Allowance of the claims is thereby respectfully solicited.

If Examiner Seharaseyon believes it to be helpful, he is invited to contact the undersigned representative by telephone at 312-913-0001.

Respectfully submitted,
McDonnell Boenken Hulbert & Berghoff LLP

Dated: December 23, 2008

By: /Donald L. Zuhn, Jr./
Donald L. Zuhn, Jr., Ph.D.
Reg. No. 48,710

Exhibit A

	10	20	30	40	50	60
SEQID2	MSTKPD	MIQKCL	WLEILMG	IFIA	GTLSL	DCNLLNVHLRRVTWQNL
SEQID5	MSTKPD	MIQKCL	WLEILMG	IFIA	GTLSL	DCNLLNVHLRRVTWQNL

Prim.cons.	MSTKPD	MIQKCL	WLEILMG	IFIA	GTLSL	DCNLLNVHLRRVTWQNL
	70	80	90	100	110	120
SEQID2	RENIA	FELPQ	EFLQY	TPMKR	DIKKA	FYEMSLQAFNIFSQHT
SEQID5	RENIA	FELPQ	EFLQY	TPMKR	DIKKA	FYEMSLQAFNIFSQHT

Prim.cons.	RENIA	FELPQ	EFLQY	TPMKR	DIKKA	FYEMSLQAFNIFSQHT
	130	140	150	160	170	180
SEQID2	QAEYLN	QCLEED	ENENED	MDKEM	KENEMK	PSEARVPQLSSLELR
SEQID5	QAEYLN	QCLEED	ENENED	MDKEM	KENEMK	PSEARVPQLSSLELR

Prim.cons.	QAEYLN	QCLEED	ENENED	MDKEM	KENEMK	PSEARVPQLSSLELR
	190	200				
SEQID2	CAWEIV	RVEIRRC	LYFYK	FTAL	FRRK	
SEQID5	CAWEIV	RVEIRRC	LYFYK	FTAL	FRRK	

Prim.cons.	CAWEIV	RVEIRRC	LYFYK	FTAL	FRRK	

Exhibit B

	10	20	30	40	50	60
SEQID2	MSTKPDMIQKCLWLEILMGIFIAGTSLDCNLLNVHLRRVTWQNLRLHLSMSNSFPVECL					
SEQID6	-----CNLLNVHLRRVTWQNLRLHLSMSNSFPVECL					

Prim.cons.	MSTKPDMIQKCLWLEILMGIFIAGTSLDCNLLNVHLRRVTWQNLRLHLSMSNSFPVECL					
	70	80	90	100	110	120
SEQID2	RENAFELPQEFLOYTQPMKRDIKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ					
SEQID6	RENAFELPQEFLOYTQPMKRDIKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ					

Prim.cons.	RENAFELPQEFLOYTQPMKRDIKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ					
	130	140	150	160	170	180
SEQID2	QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYS					
SEQID6	QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYS					

Prim.cons.	QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYS					
	190	200				
SEQID2	CAWEIVRVEIRRCLYYFYKFTALFRRK					
SEQID6	CAWEIVRVEIRRCLYYFYKFTALFRRK					

Prim.cons.	CAWEIVRVEIRRCLYYFYKFTALFRRK					

Exhibit C



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 99-372-F)

PATENT

In re Application of: Welcher et al.

Serial No.: 09/927,850

Filed: August 10, 2001

For: Interferon-Like Molecule
and Uses Thereof

Before the Examiner: J. Andres

Group Art Unit: 1646

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION ON PURSUANT TO 37 C.F.R. § 1.131

We, Andrew A. Welcher, residing at 1175 Church Street, Ventura, California; Duanzhi Wen, residing at 3885 Campus Drive, Thousand Oaks, California; and Michael Kelley, residing at 3866 Alta Mesa Drive, Studio City, California; hereby declare:

1. We are named co-inventors on United States Application No. 09/927,850, filed on August 10, 2001.
2. The invention disclosed and claimed in the instant patent application was conceived in the United States by us before July 21, 1998 and was then diligently reduced to practice.
3. Accompanying this Declaration are photocopies of forty-one (41) pages from our laboratory notebook showing conception of our invention before July 21, 1998. Specifically, the photocopies of our laboratory notebook show that a genomic cloning approach was used to identify the nucleic acid sequence of human interferon-like polypeptide (*see* page 34 of laboratory notebook). Three genomic clones were identified as containing nucleic acid sequences encoding at least a portion of human interferon-like polypeptide (*i.e.*, clones 2, 6, and 7; *see* page 40). The nucleic acid sequences from these clones were isolated and then re-cloned into a suitable sequencing vector. One of the three genomic clones was determined to contain a partial nucleic acid sequence for human interferon-like polypeptide and another genomic clone (*i.e.*, clone 6) was determined to contain a full-length nucleic acid sequence for human interferon-like polypeptide (*see* page 62). The amino

acid sequence of human interferon like polypeptide was determined from the latter nucleic acid sequence.

4. The dates on the laboratory notebook pages have been redacted from the photocopies. However, the dates e before July 21, 1998, the date on which U.S. Provisional Application No. 60/093,643 was f d, from which U.S. Application No. 09/487,792 claims the benefit of priority, from which U. Patent No. 6,433,145 issued on August 13, 2002.

5. Also accompanying this Declaration are photocopies of ten (10) pages from a Research Summary showing tha the invention disclosed and claimed in the instant patent application was diligently reduce to practice. Specifically, the photocopies of the Research Summary show that experiments ere performed in order to determine the function of protein encoded by the nucleic acid sequer e described in paragraph 3 above, and that once the function of the protein had been determined, Research Summary was prepared and submitted to the legal department of Amgen Inc., the assi ee of the instant application. More particularly, photocopies of the Research Summary show tha several versions of the human and rat IFN-L proteins were produced in a mammalian expres on system (see page 7) and that rat IFN-L:Fc fusion protein treatment of several cell lines wa found to cause phosphorylation of cellular proteins (see page 10).

6. The dates on th Research Summary pages have been redacted from the photocopies.

7. We hereby declare urther that all statements made herein by each of us to our own knowledge are true and that all st ements made on information and belief are believed to be true; and further that these statements v re made with the knowledge that willful false statements and the like so made are punishable by fi or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that su l willful false statements may jeopardize the validity of the application or any patent issuing ercon.

Dated: June 10, 2004

Signed:

Andrew A. Welcher
Andrew A. Welcher

Duanzhi Wen
Duanzhi Wen

Michael Kelly
Michael Kelly



UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 99-372-F)

PATENT

In re Application of: Welcher et al.

Serial No.: 09/927,850

Filed: August 10, 2001

For: Interferon-Like Molecules
and Uses Thereof

Before the Examiner: J. Andres

Group Art Unit: 1646

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION PURSUANT TO 37 C.F.R § 1.131

We, Andrew A. Welcher, residing at 1175 Church Street, Ventura, California; Duanzhi Wen, residing at 3885 Campus Drive, Thousand Oaks, California; and Michael Kelley, residing at 3866 Alta Mesa Drive, Studio City, California; hereby declare:

1. We are named co-inventors on United States Application No. 09/927,850, filed on August 10, 2001.

2. The invention disclosed and claimed in the instant patent application was conceived in the United States by us before July 21, 1998 and was then diligently reduced to practice.

3. Accompanying this Declaration are photocopies of forty-one (41) pages from our laboratory notebook showing conception of our invention before July 21, 1998. Specifically, the photocopies of our laboratory notebook show that a genomic cloning approach was used to identify the nucleic acid sequence of human interferon-like polypeptide (*see* page 34 of laboratory notebook). Three genomic clones were identified as containing nucleic acid sequences encoding at least a portion of human interferon-like polypeptide (*i.e.*, clones 2, 6, and 7; *see* page 40). The nucleic acid sequences from these clones were isolated and then re-cloned into a suitable sequencing vector. One of the three genomic clones was determined to contain a partial nucleic acid sequence for human interferon-like polypeptide and another genomic clone (*i.e.*, clone 6) was determined to contain a full-length nucleic acid sequence for human interferon-like polypeptide (*see* page 62). The amino

acid sequence of human interferon-like polypeptide was determined from the latter nucleic acid sequence.

4. The dates on the laboratory notebook pages have been redacted from the photocopies. However, the dates are before July 21, 1998, the date on which U.S. Provisional Application No. 60/093,643 was filed, from which U.S. Application No. 09/487,792 claims the benefit of priority, from which U.S. Patent No. 6,433,145 issued on August 13, 2002.

5. Also accompanying this Declaration are photocopies of ten (10) pages from a Research Summary showing that the invention disclosed and claimed in the instant patent application was diligently reduced to practice. Specifically, the photocopies of the Research Summary show that experiments were performed in order to determine the function of protein encoded by the nucleic acid sequence described in paragraph 3 above, and that once the function of the protein had been determined, a Research Summary was prepared and submitted to the legal department of Amgen Inc., the assignee of the instant application. More particularly, photocopies of the Research Summary show that several versions of the human and rat IFN-L proteins were produced in a mammalian expression system (*see* page 7) and that rat IFN-L:Fc fusion protein treatment of several cell lines was found to cause phosphorylation of cellular proteins (*see* page 10).

6. The dates on the Research Summary pages have been redacted from the photocopies.

7. We hereby declare further that all statements made herein by each of us to our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: June 25, 2004

Signed: _____
Andrew A. Welcher

Duanzhi Wen

Michael Kelly

Project No. _____

Book No. _____ TITLE

IFN

34

ge No. _____

hnp3-00078-F6.

① related to IFN β (32%).

② In certain lot of pancreas mRNA (human) Northern (J. Cao).

③ Screening of pancreas cDNA (human) Library.

? Different lot of RNA source

? Different level of expression

Decision: ① re-screening human pancreas library
 ② determine the genomic screening condition

③ Is genomic screening feasible?

IFN probe
 for genomic blot

PAGE: 1

13:16

ID: CHERENCOV

0.5

JSER: 2

COMMENT:

PRESET TIME :

0.50

DATA CALC :

CPM

H# : NO

SAMPLE REPEATS :

1

PRINTER

: STD

COUNT BLANK :

NO

IC# : YES

REPLICATES :

1

RS232

: OFF

TWO PHASE :

NO

AGC : NO

CYCLE REPEATS :

1

SCINTILLATOR:

XTAL

LUMEX: NO

LOW SAMPLE REJ:

0

LOW LEVEL :

NO

HALF LIFE CORRECTION DATE:

none

ISOTOPE 1:

32P

XERROR: 0.00

FACTOR: 1.000000

BKG. SUB:

0

SAM PGS

TIME - IG#

32P

LUMEX

ELAPSED

NO

MIN

CPM XERROR

%

TIME

1 ** -1

0.50 655.1

427775.8

0.43

0.00

0.83

4.3x10⁵ cpm/μl

To Page No. _____

Page No. _____

PCR-Hot rIFN probe: template 1λ
 1795-01 20pm
 1795-02 1λ
 10X PCR Buf. 10 λ
 10mM dNTPs 10 λ
 32 P-dCTP 5 λ
 25mM MgCl₂ 16 λ
 Tag 1 λ
 H₂O to 100 λ

94°C 30sec, 50°C 30sec 74°C 1min for 40 cycles.

G-50 column purified.

count: 4.3×10^5 cpm/ λ .

Determine the hybridization and washing condition for genomic screening.

- ① very likely the homology of hIFN vs. rIFN is in the neighborhood of G_{ap}.
- ② The formamide should be between 25% - 30%

washing condition should start with gentle wash, then elevate the T wash. determine a condition in which fragment is detectable while background is minor.

To Page No. _____

From the genomic southern (D. Wen keeps all the photo) it is clear:

- ① there is an 1.8kb $HindIII$ fragment that strongly hybridize w/ Probe.
- ② The formamide concentration can be adjusted to $\sim 30\%$ for relatively optimal signal vs. noise ratio.
- ③ Washing can be conducted @ $\sim 55^\circ C$ in 0.2 or 0.3X SSC, 0.1% SDS.

Wen further found in literature:

- ① IFN α family member is single gene, i.e. no intron.
- ② A lot of pseudo IFN gene as well.

But what about we identify a gene resemble mRPE3-0078-F6, also proved its expressing tissues?

Page No. _____

Screening hPancreas Library w/ Act Probe

IFN - probe for human pancreas Library screening

PAGE: 1

ID: CHERENCOV 0.5

USER: 2

COMMENT: .

10:24

PRESET TIME :	0.50						
DATA CALC :	CPM	H#	: NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC#	: YES	REPLICATES	: 1	RS232	: OFF
TWO PHASE :	NO	AGC	: NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX:	NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE	CORRECTION DATE:	none			

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM	POS	TIME	IC#	32P	LUMEX	ELAPSED
NO		MIN		CPM %ERROR	%	TIME
1	**1	0.50	606.2	827666.2	0.31	0.01 0.91

PCR as on p. 35.

Library screening

- 1X10⁶ clones on 20 plates.

- 30% formamide. 5xSSC. 42°C 9/N

- Wash: 2xSSC. 0.1% SDS 30min @ 50°C

- O/N exposure

And

There is no double positive clones.

To Page No. _____

Project No. _____

Book No. _____

TITLE

ITN

38

e No. _____

Screening human fibroblast genomic Library.

Library: human lung fibroblast genomic library in

Fix II vector (Stratagene).

 1×10^6 independent clones on 10 nitro-cellulose membrane.

PCR probe: as described on p.35

198
ITN probe for genomic Screening

PAGE: 1

12:47

D: CHERENCOV

0.5

ER: 2

COMMENT:

RESET TIME: 0.50

ITA CALC: CPM

UNT BLANK: NO

JO PHASE: NO

INTILLATOR: XTAL

JW LEVEL: NO

H#: NO

IC#: YES

AQC: NO

LUMEX: NO

HALF LIFE

SAMPLE REPEATS: 1

REPLICATES: 1

CYCLE REPEATS: 1

LOW SAMPLE REJ: 0

CORRECTION DATE:

PRINTER

RS232

STD

OFF

none

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

AM	POS	TIME	IC#	CPM	%ERROR	LUMEX	%	ELAPSED	TIME
JO		MIN							

1	**	-1	0.50	632.0	704931.5	0.34	0.01	0.89	
---	----	----	------	-------	----------	------	------	------	--

To Page No. _____

Page No. _____

Lift Library to Nitrocellulose Membrane

① 5 min denature, 5 min neutralization. 1/5 Sec 2X SSC

② 80°C bake 1 hr.

③ Pre-hyb: 30% Formamide
5X SSC
2X Denhart's
10 µg/ml ssDNA
0.2% SDS
2 mM EDTA
0.1% Pyrophosphate

42°C o/n

④ Wash:

At R-T: 1X SSC, 0.1% SDS. 2x 30 min

At 55°C 0.2X SSC, 0.1% SDS 15 min

~80°C BioMax Kodak film o/n at -80°C

Project No. _____

Book No. _____

TITLE _____

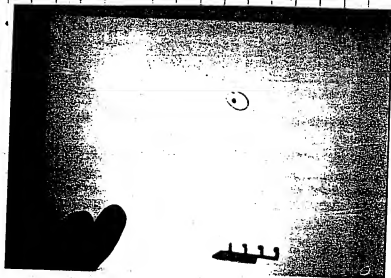
ITN

40

je No. _____

Primary screening Result.

①



#2

#6

②

I pick up the ϕ colony with
SM elution 37°C 2 hrs.

② $1/500$ dilution for secondary

③ double-lift secondary
screening membrane.

#7. All double positive

Page No. _____

2nd screening 5 6 IZN

4/19/98
IZN probe

PAGE: 1

ID: CHERENCOV 0.5

USER: 2

COMMENT:

20:13

PRESET TIME : 0.50

DATA CALC : CPM

COUNT BLANK : NO

TWO PHASE : NO

SCINTILLATOR: XTAL

LOW LEVEL : NO

H# : NO SAMPLE REPEATS: 1

IC# : YES REPLICATES : 1

AQC : NO CYCLE REPEATS : 1

LUMEX: NO LOW SAMPLE REJ: 0

HALF LIFE CORRECTION DATE:

PRINTER : STD

RS232 : OFF

none

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	%ERROR	LUMEX %	ELAPSED TIME
--------	-----	----------	-----	---------	--------	---------	--------------

1	**1	0.50	615.7	458314.7	0.42	0.01	0.86
---	-----	------	-------	----------	------	------	------

Hybridization: 30% formaldehyde
5xSSC
42°C O/N

Wash: 1xSSC, 0.1% SDS R.T. 30min

0.2xSSC, 0.1% SDS 55°C 15min

To Page No. _____

Project No. _____

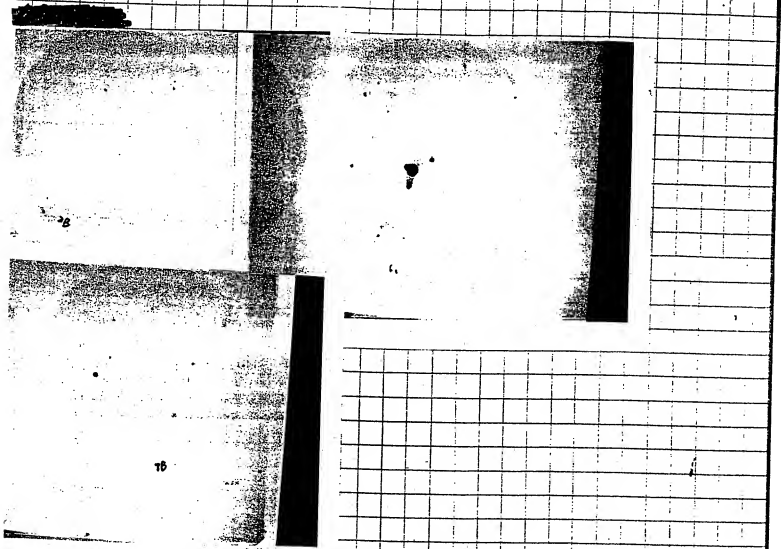
Book No. _____

TITLE _____

IAN

42

ge No. _____



Pick-up 2nd clone. elute with SM.

prepare for phage DNA preparation.

To Page No. _____

n Page No. _____

Φ DNA preparation protocol

aration of lamdan phage DNA
 row up the phage (on plates or in liquid medim).
 1lecta the supernatant and spin down with 3000rpm.
 dd DNaseI (10u/ml) and RNase(20u/ml) incubate 30 min at
 c degree.
 d 1/3 volume 30%PEG 6000 in 3M NaCl put in ice for 1 hr or at 4
 degree overnight.
 14000g centrifugation 20 min
 discard supernatant and interverte the tube on papers to remove
 G completely.
 d 1/10-1/5 of origin volume T10E1 to suspend the phage
 ticles.
 d 0.5M EDTA to a final concentration of 20mM and proteinaseK
 mg/ml, incubate 1hr in 55c degree waterbath.
 id 5%CATS in 0.5M NaCl to a final concentration of 0.1% and put
 65c degree for 5 min.
 in down and dissolve in 1.2M NaCl.
 add 2.5vol. of ethanol and spin down.
 discard the ethanol and wash once with 70% ethanol.
 r dry the pellets and dissolve in T10E1 in ice 30 min. then at
 ic degree for 10min. don't put on RT. stock in 4c degree.

- phage has to be amplified once before proceeding to Φ DNA prep. on a 100mm plate. 10u^l Φ + top agar → o/n 37°C.
- SM elute
- on LB/agarose plate, grow Φ o/n @ 37°C
- elute w/ SM 2 1/2 hrs @ 7-7.

To Page No. _____

Project No. _____

Book No. _____

TITLE _____

IFN

44

No. _____

BECKMAN DU-600

Human Genomic IFN like clone
Φ prep.Date: _____
Time: 23:19leic Acid
adSamples

Method

SaveClear

Print

Quit

Results file: A:\WORK_RES

Method name: A:\DEFAULT

Assay type: General Ratio and Concentration

Formula setup: VIEW

Sampling device: None

Read average time: 0.50 sec

Units: UG/UL

Background Correction: [No]

Concentration: [Yes]

Peak Pick: [No]

Sample	abs 260.0 nm	abs 280.0 nm
#2	0.1428	0.0774
#6	0.3206	0.1775
#7	0.1731	0.0913

260.0 nm	280.0 nm	x100 ssRNA UG/UL	X100 dsDNA UG/UL
280.0 nm	260.0 nm		
1.8453	0.5419	0.5711	0.7139
1.8066	0.5535	1.2823	1.6029
1.8962	0.5274	0.6922	0.8653



#2: 142 ng/λ

← 32 kb

#6: 32.1 ng/λ

#7: 17.3 ng/λ

Set-up NotI digestion to
release insert in FIX II.

20 μg Φ DNA

10x H

NotI (HC) 40u x 2.5 = 100

hho

ON @ 37°C incubator

To Page No. _____

IFN

Project No. _____

Book No. _____

48

Page No. _____

~~where is the IFN+ fragment?~~

The two ← indicated on p. 44 insert of interest?

Repeat Not I digestion; run a small agarose 0.5% gel.

Transfer and Southern to determine the band

Hyb: 30% formamide, wash: 0.2xSSC, 0.1% SDS.
55°C 15 min.

Results on p. 46

BECKMAN DU-600

Date: _____
Time: 16:18Nucleic Acid
Read Samples

Method

Save/Clear

Print

Quit

Results file: A:\WORK_RES

Method name: A:\DEFAULT

Assay type: General Ratio and Concentration

Units: UG/UL

Formula setup: VIEW

Background Correction: [No]

Sampling device: None

Concentration: [Yes]

Read average time: 0.50 sec

Peak Pick: [No] ↑ ↑

1/20				260.0 nm	280.0 nm	x100	X100
Sample	abs	abs				ssRNA	dsDNA
ID	260.0 nm	280.0 nm		280.0 nm	260.0 nm	UG/UL	UG/UL
1 #2	0.0059	0.0027		2.1876	0.4571	0.0235	0.0293
2 #6	0.0082	0.0043		1.8895	0.5292	0.0326	0.0408
3 #7	0.0085	0.0046		1.8522	0.5399	0.0338	0.0423
4							

#2 5.2 x 50 x 20 = 618/2
 #6 8.2 x 1000 = 818/2
 #7 8.5 x 1000 = 8.518/2

Ligation: 1018 cut vect.
 + 62 Insert
 + 22 10x Buff
 5U T4 ligase

Assessed & Understood by me,

Date

Invented by

Date

Page No. _____

[REDACTED]



[REDACTED] Gel purifying ~~ITN~~ genomic fragment

267

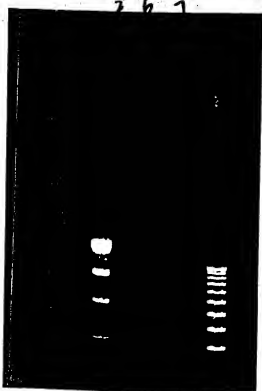


IMAGE SIZE 1448 x 488 x 81.
 INT. RESOLUTION 6.35 SEC.
 ACQUIRED [REDACTED]
 STRONGHOLD EOLLE PVE II [REDACTED] 1318219



IMAGE SIZE 1448 x 488 x 81.
 INT. RESOLUTION 6.35 SEC.
 ACQUIRED [REDACTED]
 STRONGHOLD EOLLE PVE II [REDACTED] 1318219

d & Understood by me.

Date

Invented by

[Signature]

Date

Page No. _____

PSV. SPONT/IR genomic fragment

BECKMAN DU-600

oleic Acid
adSamples

Method

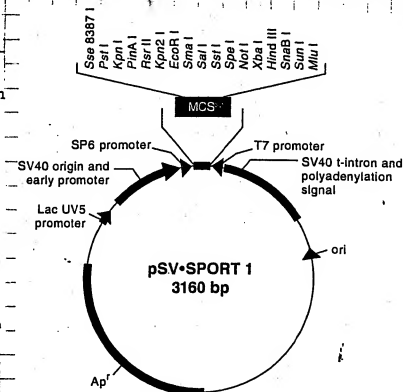
Results file: A:WORK_RES

Assay type: General Ratio and Concn
 Formula setup: VIEW
 Sampling device: None
 Read average time: 0.50 sec

Sample ID	abs	abs
	260.0 nm	280.0 nm

1 psu.pspont	0.0107	0.0039
2 psu.pspont	0.0204	0.0093
3 psu.pspont		

psu.pspont/NotI 20 ng/μl



0.8% TAE - agarose gel / NotI digestion

STRATAGENE EAGLE EYE II 12:46:39
 SIZE (640 × 480 × 8)
 BRID = 0.13 SEC.
 RES



miniprep DNA 5μl

10X H 1μl

NotI 0.5μl (10U)

H₂O 3.5μl

Total 10μl @ 37°C 1 1/2 hrs

To Page No. _____

Project No. _____

Book No. _____

TITLE _____

101 27N

48

ge No. _____

Set-up genomic clone Analysis.

5 λ mini-prep DNA
 1 λ 10 \times Buffer (BMB)
 0.5 λ EZ
 3.5 λ H₂O

Restriction E₂s.

present on MCS of psu.pspart

NotI, EcoRI, XbaI & HindIII

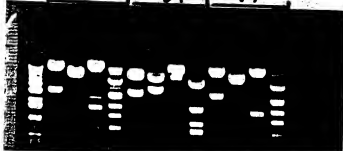
10 λ @ 37 $^{\circ}$ C 1 1/2 hrs.

87% TAE gel.

STRATAGENE EAGLE EYE II 15:10:42

548 x 488 x 81,
 0.46 SEC.

22 61 71



22 and 71 are identical independent clones. but not 61.

22 61 71

EcoRI 0 2 0

XbaI 3 2 3

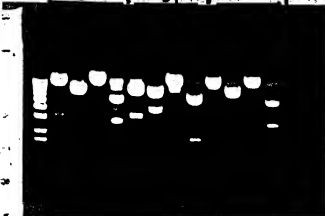
HindIII 24 24 24

Is that why initially EcoRI digestion
 on genomic DNA didn't light up
 by p.mrep3 probe?

STRATAGENE EAGLE EYE II 15:33:05

548 x 488 x 81,
 0.66 SEC.

22 61 71



NEX H NEX H

Transfer this gel w/ alkaline transfer.

22 61 71



* HindIII incomplete digestion

To Page No. _____

Page No. 27N

Washing Southern Blot.

Hybridization: 30% Formamide, 5x SSC, 2x Denhart's
~~10% SSDNA~~, 0.2% SDS, 2 mM EDTA, 0.1% propylene
 (No NH_2PO_4)
 42°C, 3 hrs. + 5% Dextran Sulfate for Hyb.

PCR - Hot probe: Template 1 λ (20ng)
 1795-01 1 λ (20pm)
 1795-02 1 λ (20pm)
 10 mM dNTP 10 λ (dCTP @ 0.1 mM)
 ^{32}P -dCTP 5 λ
 10x PCR buf. 10 λ
 25 mM MgCl_2 16 λ (4 mM final)
 Taq 1 λ
 H₂O to 100 μl Control: + 60 μl H₂O
 with 10 mM dNTP.

5/7/98 27N probe

PAGE: 1

ID: CHERENCOV

0.5

USER: 2

COMMENT:

22:04

PRESET TIME :	0.50				
DATA CALC :	CFM	H# :	NO	SAMPLE REPEATS:	1
COUNT BLANK :	NO	IC# :	YES	REPLICATES :	1
TWO PHASE :	NO	AGC :	NO	CYCLE REPEATS :	1
SCINTILLATOR:	XTAL	LUMEX:	NO	LOW SAMPLE REJ:	0
LOW LEVEL :	NO	HALF LIFE	CORRECTION DATE:	none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	%ERROR	LUMEX %	ELAPSED TIME
1	** -1	0.50	592.9	528289.8	0.39	0.01	0.86

1 ml Count.

Washing: 1 x SSC, 0.1% SDS @ RT 1 hr
 0.2 x SSC, 0.1% SDS @ 55°C 15 min
 exp @ -80°C O/N.

To Page No. 27N

Project No. _____

Book No. _____

TITLE _____

27N

50

ie No. _____

h mrep-3 cloning: Subclone genomic fragment.
 Set-up digestion: No. 61

70 λ mini-prep DNA8 λ 10x B Buffer (BMB)2 λ BamHI (20u)80 λ total @ 37°C 5 hrs8 λ run gel (with dye)

indicates positive band in
 Southern Blot with mrep-3
 probe (p. 49)

92 λ (with dye) run 0.8% TAE gel

No. 71

Qiagen Gel Purification Kit

0.8% TAE-agarose
gel

(hind III fragments)

BIOCHAM DU-600

Date: _____

Time: 21:25

leic Acid

adSamples

Method

SaveClear

Print

Quit

Results file: A:\WORK_RES

Method name: A:\DEFAULT

Assay type: General Ratio and Concentration

Formula setup: VIEW

Sampling device: None

Avg average time: 0.50 sec

Units: UG/UL

Background Correction: [No]

Concentration: [Yes]

Peak Pick: [No]

Y10 d:lu
 sample abs / abs
 260.0 nm 280.0 nm

260.0 nm 280.0 nm x100 X100
 ssRNA dsDNA
 280.0 nm 260.0 nm UG/UL UG/UL

#61 0.0168 0.0093
 #71 0.0201 0.0112

1.7933 0.5576 0.0670 0.0838
 1.7963 0.5567 0.0803 0.1003

#61 0.0168 x 50 x 20 \approx 1718 λ #71 0.0201 x 50 x 20 \approx 2013 λ

To Page No. _____

& Understood by me.

Date

Invented by

Page No. _____

Ligation:

3 λ dephosphorylated psv-pspout (17ng)
 1 λ 10x Lig. Buffer (BMB)
 1 λ T4 DNA Ligase (BMB)
 5 λ Insert (85 ~ 100ng)
 10 λ

Control: + 5 μ l H₂O in vector control.

14°C 1 hr

Transformation: 140 λ DH10d ElectroMax cell
 + 1.5 λ Ligation Mix

STRATAGENE EAGLE EYE II 13:20:23

E SIZE (440 x 480 x 81).
 PERIOD = 0.36 SEC.
 USED

Hind III

61

72

6 transformation clones/each
for mini-prep.

Digest w/ Hind III

5 λ DNA1 λ 10x B (BMB)0.5 λ Hind III (5u)3.5 λ H₂O

10x total 37°C 1 1/2 hrs

app. 7AE gel

All contain insert. (1.8 Kb)

Further Analysis. w/

KpnI, pstI, SalI (BMB)

5 λ DNA1 λ 10x Buffer0.5 λ EZ (5u)3.5 λ H₂O

10x 37°C 1/2 hr

So no additional EZ cut in the insert.

To Page No. _____

Project No. _____

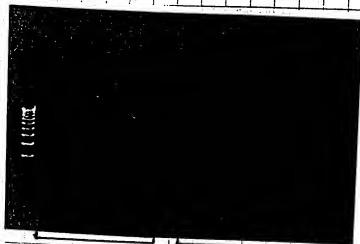
Book No. _____

TITLE _____

271

52

age No. _____



Transfer to N.C. for future reference

61

71

Sequencing Request

D	Clone	Requestor		Status	Submit	Rcvd
9808902	hgmrep3-6.1	Wen	Chen	Pending		00/00/00
9808903	hgmrep3-6.2	Wen	Chen	Pending		00/00/00
9808904	hgmrep3-6.5	Wen	Chen	Pending		00/00/00
9808905	hgmrep3-7.1	Wen	Chen	Pending		00/00/00
9808906	hgmrep3-7.2	Wen	Chen	Pending		00/00/00
9808907	hgmrep3-7.2 X deleted!	Wen	Chen	Pending		00/00/00
9808908	hgmrep3-7.3	Wen	Chen	Pending		00/00/00

3-6.1 } identical insert in same orientation
 3-6.5 }
 3-7.1 }

3-6.2 } identical insert in reverse orientation,
 3-7.2 }

Page No. _____

mCamp2 Southern: Human Genomic DNA

probe: mCamp2 template 2 λ (long)
 1.082-15 (5' primer) 2 λ (20pm)
 1.536-79 (3' primer) 2 λ (20pm)
 10x Buffer 10 λ
 10mM dNTP (dCTP @ 0.1mM) 10 λ
 α -³²P dCTP 5 λ
 25mM MgCl₂ 16 λ
 Tag (BME) 1 λ
 H₂O 52 λ

Cold control + ~~10~~ 10 λ 10mM dNTP + 52 extra H₂O
 94°C 30sec \rightarrow 60°C 30sec \rightarrow 45 Cycle \rightarrow 1min

PAGE: 1

ID: CHERENCOV 0.5

USER: 2

COMMENT:

16:06

PRESET TIME :	0.50				
COUNT BLANK :	CPM	H# :	NO	SAMPLE REPEATS:	1
DATA CALC :		IC# :	YES	REPLICATES :	1
TWO PHASE :	NO	AGC :	NO	CYCLE REPEATS :	1
SCINTILLATOR:	XTAL	LUMEX :	NO	LOW SAMPLE REJ:	0
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:	none		

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	%ERROR	LUMEX %	ELAPSED TIME
1	**1	0.50	602.8	1115364	0.27	0.02	0.95

1x 1.1 x 10⁶ cpm/1 λ

Cold control.

G/ primary Screening:



IMAGE SIZE: 1448 x 825 = 81
 INT. PERIOD = 0.04 sec.
 ACQUIRED

STRONGHOLD EAGLE B.

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Project No. _____

Book No. _____

TITLE _____

Chy 22

54

e No. _____

Hybridization in 30% Formamide

30% Formamide
5xSSC

2x Denhart's

10⁶ B/ml ssDNA

0.2% SDS

2mM EDTA

0.1% Pyrophosphate

H₂O

to 100 ml

Stock
30ml
5ml

4ml

1ml

1ml

0.4ml

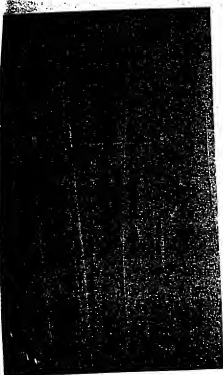
1ml

3.6ml

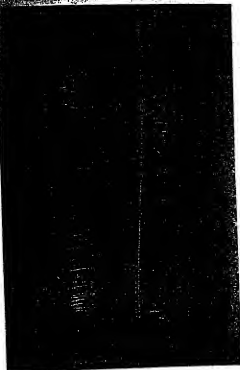
420 C 2N. stripe rehyb



20% Formamide, 0.5% Sarkosyl NL30, 24 hrs
IMAGE SIZE (400 x 400 x 81)
REAL-TIME ACQUIRE



20% Formamide, 0.5% Sarkosyl NL30, 24 hrs
IMAGE SIZE (400 x 400 x 81)
REAL-TIME ACQUIRE



20% Formamide, 0.5% Sarkosyl NL30, 24 hrs
IMAGE SIZE (400 x 400 x 81)
REAL-TIME ACQUIRE

STANDARD SCALE EYE 11 198730

ation 30%
AC (Formamide)
SSC 0.2%
SDS 0.1%
c exp 24 hrs

20%
0.5%
0.1%
24 hrs

20%
0.5%
0.1%
24 hrs

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E

IFN

Project No. _____

Bank No. _____

5

REVERSE-COMPLEMENT of: 9808902.Con check: 3852 from: 1 to: 1163

WChen

DNA-hgmrep3-6.1 p=890-24 end

assembled by JK: [REDACTED]

9808902.con

With 1 enzymes: HINDIII

[REDACTED] 17:01 ..

CTGAGAAGAGTCACCTGGCAAATCTGAGACATCTGAGTAGTATGACGAATTCATTTCCT
1-----+-----+-----+-----+-----+-----60
GACTCTCTCAGTGGACCGTTTATGACTCTGTGACTCATCTACTCTGTAAAGTAAGGA

a L R R V T W Q N L R H L S S M S N S F P -
GTAGATGTICTACGAGAAACATAGCTTTTGTAGTGTCCCAAGAGTTTCTGCAATACACC
61-----+-----+-----+-----+-----+-----120
CATCTTACAGATGCTCTTTTGTATCGAAACTCAACGGGGTCTCAAGAAGTTATGTGG

a V E C L R E N I A F E L P Q E F L Q Y T -
CAACCTATGAAGAGGGACATCAAGAAGGCCCTCTATGAATGTCCCTACAGGCTTCAAC
121-----+-----+-----+-----+-----+-----180
GTGGTACTTCTCCCTGTAGTCTTCCGGAAGATACTTTACAGGGATGTCCGGAAGTTG

a Q P M K R D I K K A F Y E M S L Q A F N -
ATCTTCAGCCCAACACACCTTCAAATATTTGGAAGAGAGACACCTCAAACAAATCCAAATA
181-----+-----+-----+-----+-----+-----240
TAGAAGTCGGTTGTGTGGAAGTTTATAACCTTTCCTCTCIGTGGAGTTTGTTTAGGTTTAT

a I F S Q H T F K Y W K E R H L K Q I Q I -
GGACTTGATCAGCAAGCAGAGTAACTGAACCAATGCTTGGAGGAGCAGCAATGAAAT
241-----+-----+-----+-----+-----+-----300
CCTGAAGTACTGCTTGCTCATGGACTTGGTTACGAACCTCCTCTCIGCTCTTACTTTTA

a G L D Q Q A E Y L N Q C L E E D E N E N -
GAAGACATGAAGCAATGAAGAGAAATGAGATGAAACCTCAGAGCCAGGGTCCCCAG
301-----+-----+-----+-----+-----+-----360
CTCTGTACTTTCCTTACTTTCCTTACTTACTTGTGGAGCTCTCGGTCCACGGGGTC

a E D M K E M K E N E M K P S E A R V P Q -
CTGAGCAGCCTGGAAGTGAAGATATTTTCCAGGATAGACAATTTCTGAAAGAAAAG
361-----+-----+-----+-----+-----+-----420
GACTGTGCGCACTTACTCTCTATAAAGGTGCTCTATCTGTATAAGGACTTTCCTTTTC

a L S S L E L R R Y F H R I D N F L K E K -
AAATACAGTGAAGTGTGCTGGGAGATTGTCCGAGTGGAAATCAGAAGATGTTTGTATTAC

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Date _____

TITL 5

IFN

56

TTTATGTCAC TGACACGGACCCCTCTAACAGGCTCA CCTTTAGICTTCTACAAACATAATG

K Y S D C A W E I V R V E I R R C L Y Y -

481 TTTTACAAATTTACAGCTCTATTTCAGGAGGAAATAAGGTATATTTTTTGGAAATTAAATTC 540

AAAATGTTTAAATGTCGAGATAAGTCTCCTTTATTCATATAAAAACTTAATTTTAAAG

F Y K F T A L F R R K * G I F L E L K F -

541 CTTTTCCTCCGAAATCTCTTCTCCTCTCCTCCTCCATCTTCTTTTAAAGGATTGTG 600

GAAAAGGGAGGCTTTAGAGAAAGAGGAAGAGGAGGAGGTAGAAGAAAAATTCTTACAAC

L F P P K S L S P S P P P S S F * G L L -

601 TGCTGTCTCTGTAGGCTGTCTCAGTTGGACTGGTAGGCTGGGAACATCAGGGACACTCA 650

ACGACAGGACATTCGGACAGGAGTCAACCTGACCATCGGAGCCTTGTAGTCCCTGTGAGT

C C P V S L S S V G L V A S E H Q G H S -

661 CCTCTCTAAGGAGAGGTAATGCCAACCATCCTCAGGGTGACCAAGAGTCTCTCTTAGAAG 720

GGAGAGATTCTCTCCATTACGGTTGGTAGGAGTCCCACTGGTTCCTCAGAGGAATCTTTC

P L * G E V M P T I L R V T K S L L R K -

721 TCTTTAAGACATTTTTAAAGGAATAAGATTCCCTCTCCGTCCTCTCTATTCTCTCTTGC 780

AGAAATTCGTAAAAATTTCTTATTCTAAGGGAGAGGCAGAAGAAGATAACAGAGAACG

S L R H F * R N K I P S P S S S I L S C -

781 TCTTTTCGIGGCCATTTTCAAAGAGCTTTGCTATATATACCACTGIGGACTTCACCA 840

AGAAAAGACACCGGTAAACTTTCTCGAAACGATATATATGGTGGCACTGAAGTGGTT

S F L W P F * K S F A I Y T T C G L H O -

841 GACAATGGCTAGAGGATAGGGAGCAGAGAATGTTGCAAAATGGTAACATTTCAATGACTT 900

CTGTTACCGATCTCCTATCCCTCGTCTCTTACAACGTTTACCATTGTAAAGTTACTGAA

D N G * R I G S R E C C K M V T F O * L -

901 AACTGTTTIGCTGCCAAGGTIGCTTATCCTATGAAAATTCAGCACATTAAAGAGCTTAT 960

TTGACAAAACGACGGTTCACGAATAGGATACTTTTAAGTCGIGTAATTTTCTCGAATA

N C F A A K V A Y P M K I Q H I K R A Y -

961 ACATGCTCCCTAGAGTCAATACTCTTGCATTTTCCCCCTCCTGCTCGGGGGGAAAAAGGT 102

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a T C S L E S I L L H F P P P A R G E K G -
 TGACATTTCCTGGCCCATTTTCCTCTCAGCTTGGTTTGTGTTGAATGATGCTGTGGAATG
 1021 +-----+-----+-----+-----+-----+ 1080
 ACTGTAAAGACCGGTTAAAGGAAGAGTCGAACCAACAACTTAACTACGAACACCTTAC

INTRON>

INTRON>

a * H F W P I S F S A W F V * I D A C G M -
 GTATTTCATTACTTTAAGAGTGAAGATCCATAGTGAATGGATGGATGGTGTGAATTAGA
 1081 +-----+-----+-----+-----+-----+ 1140
 CATAAAGTAATGAATTTCTCAGTTCTAGGTATCACTTTAACCCTACCTACCAACTTAATCT

a V F H Y F K S E D P * * N W M D G * I R -

HindIII

1141 +-----+-----+ 1163
 CGACCATTAGCTTTAGCTACGGG
 GCTGGTAATTCGAATGCATGGC

a R P L S L R T -

Enzymes that do cut:

HindIII

Untitled-5	MTLKYLNLVA LVALYISPIQ SQNCVYLDHT ILEMKLMSNSVE	17
Untitled-3	MTLKYLNLVA LVALYISPIQ SQNCVYLDHT ILEMKLMSNSVE	50
Consensus	50
Untitled-5	RNIATLHLSRCHLPMHSLKARVEM SLOFNLHSHITFKYWKER	66
Untitled-3	RNIATLHLSRCHLPMHSLKARVEM SLOFNLHSHITFKYWKER	100
Consensus	100
Untitled-5	HKKCHDIDQCAEYLNLCL EATLSEEM KEMKENEMSEARVPQLSS	116
Untitled-3	HKKCHDIDQCAEYLNLCL EATLSEEM KEMKENEMSEARVPQLSS	146
Consensus	150
Untitled-5	RRFHHLDNKKHSLYHFTALFRRK	164
Untitled-3	RRFHHLDNKKHSLYHFTALFRRK	191
Consensus	198

Ratio: 2.484 Gaps: 4
Percent Similarity: 63.383 Percent Identity: 63.383

Match display thresholds for the alignment(s):

| = IDENTITY

 $i = 5$ $\bullet = 1$

9808902.Con x Mrpe3-00078-F6-Wz.Ctg [REDACTED] 13:52 ..

1152 CACCTGGCAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCTG 1103

|||||

159 CATCTTGGAAAACATGAAACTTCTGAGCAGCATCAGGACCACTTTTCCCT 208

1102 TAGAATGTCCTACGAGAAAACATAGCTTTTGAGTTGCCCAAGAGTTTCTG 1053

|||||

209 TAAGATGTCCTAAAAGATATCACGGATTTTTCAGTTTCTCAAGAGATTTCIG 258

1052 CAATACACCCAAACCTATGAAGAGGGACATCAAGAAGGCCCTTCTATGAAAT 1003

| | | | | | | | | | | | | | | | | | | | | |

259 CTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACCTATCATAT 308

1002 GTCCCTACAGGCCCTTCAACATCTTCAGCC...AACACACCTTCAATATT 956

[illegible]

309 ATCTTCTGTTGGCGCTAATTATTTTTCAGTCTTTAAAGACTTCATCTTCCTGG 358

955 GGAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTTCATCAGCAAGCA 906

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	5
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359 CGACAGAGCAACCGCTTGGCAACGGTATCAGATCGGGCACTTTTCAAACAAGTG 408

905 GAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACAT 856

[illegible]

409 CAGCAAGCTCGAGAGTGCATGGTACACGAGGAGAACAGA.....ACAC 452

855 GAAAGAAATGAAAGAGAATGAGATGAAACCCCTCAGAAGCAG.GGTCCCC 807

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	5
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453 GGAGG.....AGGACAGTACATCACAACATCTTCACTCAGAGGGGCTTC 495

806 CAGCTGAGCAGCCTGGAACTGAGGAGATATTTTCACAGGATAGACAATTT 757

[illegible]

496 AAGGCAGTCTACCTTGAATTGAAACAAGTATTTCTTCAGAATCAGAAAGTT 545

756 CCTGAAAGAAAAGAAATACAGTGCATGTTGGCTGGGAGATTGTCGAGTGG 707

[illegible]

546 CTTGGTAAATAAGAAATACAGTTHHNIIGGCGTTCGAAGATCTGTCTGGTGG 595

706 AAATCAGAAGATGTTTGTATTACTTTTACAAATT 673

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	5
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596 AAATAAGAAGATGTTTCAGTATATTTTACAAACT 629

To Page No. _____

Project No. _____

Book No. _____

TITLE _____

IFN

60

lo. _____

Gaps: 4
Quality: 1170
Lity Ratio: 2.484
Similarity: 63.383
Length: 484

N> type 9808902.pair

FIT of reverse of: 9808902.Can check: 3852 from: 1 to: 1163

gmrep3-6.1 p=890-24 end

bled by JK: [REDACTED]
02.con

Mrpe3-00078-F6-Wz.Ctg check: 4485 from: 1 to: 963

INTRON::DONGYINY
WCHEN

[REDACTED] 16:07:43.44

INTRON::JCAO
DONGYINY . . .

[REDACTED] 12:24:38.19

ol comparison table: Gencoredisk: [Gogcore.Data.Rundata]Swgapdna.Cmp
heck: 2335

Gap Weight: 50 Average Match: 10.000
Length Weight: 3 Average Mismatch: -9.000

Quality: 1170

Length: 484

RIFN

Rabbit 2 RIFN

Programme	Interferon Like Protein		
Investigator:	Duanzhi Wen		
Animal/Quant	3 rabbits		
Immunogen:	Interferon like Protein		
Protocol:	MSU Standard		
Prog start date:			
Prog end date:	Ongoing		
Comments:	Give all sera to Duanzhi.		
ANIMAL# PI		50% Titer	50% Titer
#3429 5mls	30mls		30mls
#3432 5mls	30mls		30mls
#3433 5mls	30mls		30mls

Programme	Interferon Like Protein		
Investigator:	Duanzhi Wen		
Animal/Quant	3 rabbits		
Immunogen:	Interferon like Protein		
Protocol:	MSU Standard		
Prog start date:			
Prog end date:	Ongoing		
Comments:	Give all sera to Duanzhi.		
NIMAL# PI		50% Titer	50% Titer
3429 5mls	30mls	1 1500	30mls 1 2000
3432 5mls	30mls	1 2500	30mls 1 3000
3433 5mls	30mls	1 5000	30mls 1 10,000
	50% Titer		
25mls	> 1	10,000	
25mls	> 1	10,000	
25mls	> 1	10,000	

REVERSE-COMPLEMENT of: 9808903.Con check: 57 from: 1 to: 1894

WChen

DNA=hgmrep3-6.2

assembled by JK.

9808903.com

With 1 enzymes: HINDIII

15:37 ..

HindIII

[illegible]

ATCCCTGGACTGTAACTTACTGAAAGTTCACCTGAGAAGAGTCACTGGCAAAATCTGAG
 661-----+-----720
 TAGGGACCTGACATTGAATGACTTGCAGTGGACTCTTCTCAGTGGACOGTTTGTAGACTC
 ACATCTGAGTAGTATGAGCAATTCAATTTCTGTAGAATGTCTAOGAGAAAACATAGCTTT
 721-----+-----780
 TGTAGACTCATCATCTCGTTAAGTAAAGGACATCTTACAGATGCTCTTTTGTATCGAAA
 TGAGTTGCCCAAGAGTTTCTGCAATACACCCAACTATGAAGAGGGACATCAAGAAGGC
 781-----+-----840
 ACTCAACGGGGTCTCAAAGACOGTTATGTGGGTGGATACTTCTCCCTGTAGTTCTTCCG
 CTTCATGAAATGTCCCTACAGGCTTCAACATCTTCAGCAACACACCTTCAAATATTG
 841-----+-----900
 GAAGATACCTTTACAGGGATGTCCGGAAGTGTAGAAAGTGTGTGGAAAGTTTATAAC
 GAAAGAGAGACACCTCAAACAANTCCAAANTAGGACTTGATCAGCAAGCAGAGTACCTGAA
 901-----+-----960
 CTTTCTCTCTGTGGAGTTTGTATTAGGTTTATCTCGAAGTGTGTGTCTCATGGACTT
 CCAATGCTTGGAGGAGACGAGAATGAAAATGAAGACATGAAGAAGTGAAGAGAAATGA
 961-----+-----1020
 GGTTACGAACCTCTCTCTCTTACTTTTACTTCTGTACTTCTTACTTCTCTTACT
 GATGAAACCTCAGAAGCCAGGGTCCCCAGCTGAGCAGCTGGAAGTGAAGAGATATTT
 1021-----+-----1080
 CTACTTTGGAGTCTTCGGTCCAGGGGTGACTGTGTGGACCTTGACTCTCTATAAA
 CCACAGGATGACAATTTCTCGAAAGAAAAGAAATACAGTACTGTGCTGGGAGATTGT
 1081-----+-----1140
 GGGTGTCTATCTGTAAAGGACTTTCTTTTCTTTATGTCACTGACAGGACCTCTAACA
 CCGAGTGGAAATCAGAAGATGTTTGTATTACTTTTACAAATTTACAGCTCTATTACAGGAG
 1141-----+-----1200
 GGCACACTTTAGTCTCTCAAAACATAATGAAAATGTTTAAATGTGAGATAGATCTTC
 GAAATAGGATATAATTTTGGAAATTAATTTCTTTTCTCTCGAAGTCTTCTCTCTC
 1201-----+-----1260
 CTTTATTCCATATAAAAACTTAATTTTAAGGAAAAGGGAGGCTTTAGAGAAAGAGGAG
 TCTCTCTCATCTCTCTTTTAAGGATGTGTGTGCTGTCTGTAGCCTGTCTCAGTTGG
 1261-----+-----1320
 AGGAGGAGGTAGAAGAAAATTCCTAACAACAGACAGGACATTGGACAGGAGTCAACC
 ACTGGTAGCCTGGAAACATCAGGACACTCACCCTCTTAAGGAGAGGTAATGCCAACCAT
 1321-----+-----1380
 TGACCATCGGAGCTTGTAGTCCCTGTGAGTGGAGAGATTCTCTCCATTACGGTTGGTA
 CCTCAGGGTGAACAAGAGTCTCTTAGAAAGTCTTTAAGACATTTTAAAGGAATAAGAT
 1381-----+-----1440
 GGAGTCCACTGGTTCTCAGAGGAATCTTTACAGAAATCTGTAAAAATTTCTTATTTCTA
 TCCCTCTCGGCTCTCTCTATTCTCTCTGTCTCTTTCTGTGGCCATTTTGAAGAGCTT
 1441-----+-----1500

AGGGAGAGCCAGAGAAGATAAGAGAGAAAGAGAAAGACACCGGTAAAACTTTCTCGAA
 TGCTATATATACCACCTGTGGACTTCAACAGACAATGGCTAGAGGATAGGGAGCAGAGA
 1501 -----+-----+-----+-----+-----+-----+-----+----- 1560
 ACGATATATATGGTGGACACCTGAAGTGGTCTGTTCACCGATCTCCATCCCTGCTCTCT
 ATGTTGCCAAAATGGTAACATTTCATGACTTAACTGTTTTCGTCGCAAGGTGCTTATCC
 1561 -----+-----+-----+-----+-----+-----+-----+----- 1620
 TACAACGTTTTCACATTGTAAAGTTACTGAAATTGACAAAACGACGGTTCACACGAATAGG
 TATGAAAATTTCAGCACATTAAAGAGCTTATACATGCTCCCTAGAGTCAATACTCTTGCA
 1621 -----+-----+-----+-----+-----+-----+-----+----- 1680
 ATACTTTTAAAGTGTGTAATTTTCTCGAATATGTACGAGGATCTCAGTTATGAGAAGT
 TTTTCCCTCTCTGCTCGGGGGGAAAAAGGTTCACATTTTCIGGCCATTTCTCTCTCAGC
 1681 -----+-----+-----+-----+-----+-----+-----+----- 1740
 AAAAGGGGGAGGACGAGCCCCCTTTTTCACACTGTAAAGACCGGGTAAAGGAAGAGTGG
 TTGGTTTGTGTTGAATTGATGCTTGTGGAATGGTATTTTCAATTAAGAGTGAAGATCC
 1741 -----+-----+-----+-----+-----+-----+-----+----- 1800
 AACCACCAAACTTAACTACCAACACCTTACCATAAAGTAATGAAATTCACATTCIAGG

HindIII

 ATAGTGAAATTGGATGGATGGTTGAATTAGACGAACATTAAAGCTTGGATCTCTAGAGCG
 1801 -----+-----+-----+-----+-----+-----+-----+----- 1860
 TATCACTTTAACTACCTACCAACTTAATCTGCTGGTAATCGAACCTAGGAGATCTGCG
 GCGCGGACTAGTGAGCTGTGCGACCGGGGAATT
 1861 -----+-----+-----+-----+-----+-----+-----+----- 1894
 CGGCGGCTGATCACTGAGCAGCTGGGGCCCTAA

65

1810 1830 1850

27N

rat clone

10	30	50
GTGCAACCACGCGTCCGGGTGTGTAGATATTTTTCCTTTGGAAAGAAATACTGAGCACC		
70	90	110
AAGGCTGAGATGACACTGAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTICA		
MetThrLeuLysTyrLeuTrpLeuValAlaLeuValAlaLeuTyrIleSer		
130	150	170
CCCATCCAGTCTCAGAACTGTGTGATCTGGATCATACCATCTTGGAAAACATGAAACTTT		
ProIleGlnSerGlnAsnCysValTyrLeuAspHisThrIleLeuGluAsnMetLysLeu		
190	210	230
CTGAGCAGCATCAGGACCACCTTTCCCTTAAGATGCTAAAGATATCAACGGATTTTGAG		
LeuSerSerIleArgThrThrPheProLeuArgCysLeuLysAspIleThrAspPheGlu		
250	270	290
TTTCTCAAGAGATCTGCTGTACGTCCAGCATGTGAAAAAGGACATAAAGCCAGTCACC		
PheProGlnGluIleLeuLeuTyrValGlnHisValLysLysAspIleLysAlaValThr		
310	330	350
TATCATATACTCTCTGCGCTAATATTTTTCAGTCTTAAAGACTCCATCTCCCTGGCG		
TyrHisIleSerSerLeuAlaLeuIleIlePheSerLeuLysAspSerIleSerLeuAla		
370	390	410
ACAGAGGAACGCTTGGAAAGTATCAGATCGGCACCTTTTCAACAAGTGCAGCAAGCTCGA		
ThrGluGluArgLeuGluArgIleArgSerGlyLeuPheLysGlnValGlnGlnAlaArg		
430	450	470
GAGTGCATGGTAGACGAGGACAACAAGAACCGGAGGAGGACAGTACATCACAACATCCT		
GluCysMetValAspGluGluAsnLysAsnThrGluGluAspSerThrSerGlnHisPro		
490	510	530
CACTCAGAGGGCTTCAAGGCAGTCTACCTGGAATTGAACAAGTATTCTTCTCAGAATCAGA		
HisSerGluGlyPheLysAlaValTyrLeuGluLeuAsnLysTyrPhePheArgIleArg		
550	570	590
AAGTTCCTGGTAAATAAGAAATACAGTTTCTGTGCTCGAAGATTGTCTGGTGGAAATA		
LysPheLeuValAsnLysLysTyrSerPheCysAlaTrpLysIleValValValGluIle		
610	630	650
AGAAGATGTTTCAGTATATTTTACAAACTACTCAACATGAATTGAGAAATCATCCAGCTTC		
ArgArgCysPheSerIlePheTyrLysLeuLeuAsnMetAsnEnd		
670	690	710
AAGCAAGACTTAGATAGAAGTTGTGATCGCTCAAAATGTCCCAAGAACGCTTGATTCTA		
730	750	770
AGGCTATTTCGAGTCTGCTGCTACACACTTGGCAAGCAAGACTTTTCAAGGTCAGGTTTC		
790	810	830
AAGGCAGTACAGTCAAAGGAAGTCTTAAGTTAAGCAAAAAGAAAAATTTCAGTGGAAAAGC		
850	870	890
TAGCAGAAATGTCAACTGTGCAAAAAAACAACCTATTGGATTATGGCAATTCAGTTACTAG		
910	930	950
CAAAAAAATAAAACAAAAAACAACAGTCCTAAAAAATAAAAAAAGGCGCGC		

CGC

6

9808903.com

IAN

To Page No

853 GTCCCTACAGGCCCTTCAACATCTTCAGCC...AACACACTTCAAATATT 899
|| ||| || | ||| ||| ||| ||| |||
309 ATCTTCTCTGGCGCTAATTATTTTTCAGTCTTAAGAAGCTCATCTCCCTG 358

900 GGAAGAGAGACACCTCAAAACAATCCAANTAGGACTTGATCAGCAAGCA 949
|| | ||| ||| ||| ||| ||| ||| |||
359 CGACAGAGGAACGCTTGGAACTATCAGATCGGACTTTTTCAACAAGTG 408

950 GAGTACCTGAACCAATGCTTGGAGGAAGACGGAATGAAAATCAAGACAT 999
|| | ||| ||| ||| ||| ||| |||
409 CAGCAAGCTCGACAGTGTGCATGGTAGACGAGGAGAACAGA.....ACAC 452

1000 GAAAGAAATGAAAGAGAAATCAGATGAAACCTCAGAGGCGAG.GGTCCCC 1048
|| | ||| ||| ||| ||| ||| ||| |||
453 GGAGG.....AGGACAGTACATCACAACTCTCTACTCAGAGGCGCTTC 495

1049 CAGCTGACGAGCTCGAAGCTGAGGAGATATTITCCAGGATAGACAATTT 1098
|| | ||| ||| ||| ||| ||| ||| |||
496 AAGCGAGTCTACCTGGAATTGAACAAGTATTTCCTTCAGAAATCAGAAATT 545

1099 CCTCAAGAGAAAAGAAATACAGTACTGTGCTGGGAGATTGTGCGAGTGG 1148
||| ||| ||| ||| ||| ||| ||| ||| |||
546 CCGTGTAATAAGAAATACAGTTTCTGTGCTGGAGATTGTGCTGGTGG 595

1149 AAATCAGAGCATGTTTGTATTACTTTTCAAAATT 1182
||| ||| ||| ||| ||| ||| ||| ||| |||
596 AAATAAGAGAGATGTTTCAGTATATTTCACAAACT 629

ITN

Project No. _____

Book No. _____

protein sequence comp

BESTFIT of: 9808903.Pep check: 4904 from: 1 to: 201

TRANSLATE of: 9808903.rev check: 8672 from: 602 to: 1205
generated symbols 1 to: 201.

REVERSE-COMPLEMENT of: 9808903.Con check: 57 from: 1 to: 1894
WChen
DNA-hgmrep3-6.2
assembled by JK: [REDACTED] . . .

to: Mrpe3-00078-F6-Wz.Pep check: 5990 from: 1 to: 192

TRANSLATE of: mrpe3-00078-f6-wz.ctg check: 4485 from: 70 to: 646
generated symbols 1 to: 192.

From: INTRON::DONGYINY [REDACTED] 16:07:43.44
To: WCHEN
CC:
Subj: . . .

Symbol comparison table: Gencoredisk: [Gogcore.Data.Rundata]Blosum62.Cmp
CompCheck: 6430

Gap Weight: 12 Average Match: 2.912
Length Weight: 4 Average Mismatch: -2.003

Quality: 292 Length: 194
Ratio: 1.570 Gaps: 3
Percent Similarity: 49.730 Percent Identity: 40.541

Match display thresholds for the alignment(s):
| = IDENTITY
: = 2
= 1

9808903.Pep x Mrpe3-00078-F6-Wz.Pep [REDACTED] 14:55 . . .

1 MIQKCLWLEITMGIFTAGILSLDONLNVHLRRVITWNLRLHLSMSNSFP 50
| | || | . : | . | | : | : | | . ||
1 MTKYLWLVALVALYISPIQSONC....VYLDHTILENMKLLSIRITFP 46
51 VECLENTAFELPOEFLOWTQPMKRDIKKAFYEMSLQAFNIFS.QHTIFY 99
| : : | | | | : | : | | | . | | | .
47 LRCLKDTIDFEFFQETLLVQHVKKDKIKAVTYHISLLALITFSKDSISL 96
100 WKERHLKQIQIGLDQQAAYINQCLEEDENENEDMKEMKENMKPSEARVP 149
| | . | . | : : : | : | : | : | : | :
97 ATEERLERIRSGLFKQVQARECMVDEENKTEEDSTSQHPHSEGFKAV. 145
150 QLSSELELRRYFHRIDNFLKEKKYSDCAWEIVRVEIRRCLYFYFK 193
| | : | | | | | | | | | | | | | |
146 ...YLELNKYFFRIRKFLWNKYSFCAWKLVVVEIRRCFSIFYK 186

27% identity

IN

hIFN beta	M A S L Q I A V L C E T T A P M S Y N L L G F L Q R S S N F C O K -----		93
hIFN-like	T Q C W L E I M G I F L A G I N L ----- D E N L I N V H R R V I L		36
Consensus	-----		50
hIFN beta	E I N - G R L - E Y - - - K E R M A T I E E I T K L O F C E H A A L T T L Q 3		85
hIFN-like	S R H L S S M S N S F V E R E N I A I L L C F L E Y T E P M F I K K A F I S L		86
Consensus	-----		100
hIFN beta	N I P E E P S S S T G N E T I V E N L L A N V Y H Q I N E I T -----		122
hIFN-like	Q E N E I S H T F K Y - K E - - - F E I S Q I G L D Q Q A E Y L N Q		121
Consensus	-----		150
hIFN beta	----- L E E K L E K E F T E K L M E H K V Y G A L H Y E P A 157		
hIFN-like	C L E E D E N E N E D M K E M A N E M K P S E - A E V P Q L S E R S Y F H A D N E H E K 170		
Consensus	-----		200
hIFN beta	F I I L L L A V E L L N F F I N R L G Y L N -		187
hIFN-like	K E I L L L F L C L Y F Y K E P A L E R K		201
Consensus	-----		231

Untitled-5 Formatted Alignment

17% idem

rIFN-like	TINMVLVA	I VALYPIQ	D	WVY	HHTI--L		43
hIFN beta	INQLQIA	L LCFSTAL	MSYNLLGFL	QRSSNF	SOK	--L--W	97
hIFN-like	IQCHMLEI	MGIPFGTL	C	N	NL	VNHLLRRVTW	36
Consensus	IT...WL.A	...FI..L				...L--W	50
rIFN-like	IKKLLSSIR	TTFPLR	KMTTFFRI	LLYVHWK	IKAVITHISS		82
hIFN beta	IN--GRLE	-EY-	GKRMMHIS	KQLQFCE	AALTINEMLO		86
hIFN-like	INERHLSSMS	NSFPVE	RNTAF	LQYTPEP	TIKKAEFMSL		85
Consensus	IN...LSS...	...FP...	...HGH	LQY.S.	...TK...LS.		100
rIFN-like	LALIESLKD	SISLATI	---	E-E-E-R-R-S-G	LFKQVQQARE		118
hIFN beta	NIFAEDQDS	SSTGWETIV	ENLIANVYHQ	INHAKT			122
hIFN-like	QAFTESQHT	PKY-WKE	---	-R-H-K-Q-Q-I-G	LQQAQAYLNQ		121
Consensus	.AF...EQ...	S...W.E	---	-.HK...G.L.Q.....			150
rIFN-like	CMVDEEN---	--KNTEIST	SOHPHEEGFK	AVIEENKF	FPRKFMVN		163
hIFN beta	---	--LEKL	EKEDFERGKL	MSEEFKRY	GGSLHYKA		157
hIFN-like	CLEEDENE	DMKEMENEM	KPSE-TRVPQ	LSSDFRRFY	HEIDNFKEK		170
Consensus	C....EN---	--K...E...	...RG...	SSD.R.FY	...FEK.E		200
rIFN-like	K...	K...	K...	K...	K...		
hIFN beta	K...	K...	K...	K...	K...		191
hIFN-like	K...	K...	K...	K...	K...		187
Consensus	K...	K...	K...	K...	K...		201
rIFN-like	K...	K...	K...	K...	K...		
hIFN beta	K...	K...	K...	K...	K...		191
hIFN-like	K...	K...	K...	K...	K...		187
Consensus	K...	K...	K...	K...	K...		201

JFN

From Page No. _____

Chen, Wen

From: Schultz, Henry
 Sent: Friday, [REDACTED] 5:15 PM
 To: Chen, Wen
 Subject: RE: human interferon like seq.

Wen - the human predicts cytokine strongly
 scoreaacomp= -2.9 scoredipep= 19 ACCEPT (Probability 91%).

The human is predicted to be signal peptide as follows:

MIQKCLWLEILMGIFIAGTL\$ cleavage LD.....etc

For the rat, the cytokine prediction is lost but the signal peptide is:

MTLKYLWLVALVALYISPIQS cleavage QN....etc

Henry

 From: Chen, Wen
 Sent: Friday, [REDACTED] 4:02 PM
 To: Schultz, Henry
 Subject: human interferon like seq.

Henry: please help me look at the signal peptide for the human clone sequence.
 Thanks.

Wen Chen

Human sequence:

MIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFP

51 VECLRENIAPQLPQEFQYTPQMKRDIKKA FYEMSLQAFNIFSQHTFKYW

101 KERHLKQIQIGLDQQA EYLNQCLEEDENENEDMKEMKENE MKPSEARVPQ

151 LSSLELRRYFHRIDNFLKEK KYSDCAWEIVRVEIRRCLYYFYKFTALFRR

201 K

[illegible]

From Page No. _____

gLySEnd

1270 1290 1310
 TCCTCCCTCCATCTCTCTTTTAAAGATTGTGTGCTGCTCCGTAAAGCCTGCTCAGTTGG
 1330 1350 1370
 ACTGGTAGCCTGGGAACATCAGGACACTCACTCTCTAAGGAGGTAATGCAACCAT
 1390 1410 1430
 CCTCAGGGTGAACAGAGTCTCTCTTAGAAAGTCTTTAAGACATTTTAAAGGAATAAGAT
 1450 1470 1490
 TCCCTCTCGCTCTCTCTTATCTCTCTCTGCTCTCTTTCTGCTGGCCATTTTGAAGAGCTT
 1510 1530 1550
 TGCCTATATATACCACTGTGGACTTCACCAAGACATGGCTAGAGGATAGGGAGCAGACA
 1570 1590 1610
 ATGTGTCAAAATGGTAACATTTCAATGACTTAACCTGTTTGTCTGCCAAGGTTGCTTATCC
 1630 1650 1670
 TATGAAAATTTCAGCACATTAAAGAGCTTATACATGCTCCCTAGAGTCAATACCTCTTGCA
 1690 1710 1730
 TTTTCCCTCCTCTGCTGGGGGGGAAAAAGGTTGACATTCTCTGCCCCATTTCTCTCAGC
 1750 1770 1790
 TTGGTTTGTGTGAATGATGCTTTGTGGAAITGGTATTTCATTACTTTAAGAGTGAAGATCC
 1810 1830 1850
 ATAGTGAATTTGGATGGATGGTTGAATTAGACGACCATTAAGCTTGGATCCTCTAGAGCG
 1870 1890
 GCGCCGAGTACTAGTACGCTGTGTGACCGCGGAATT

0	400	800	1200	1600	2000
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ORF Map (Universal)

Frame 3

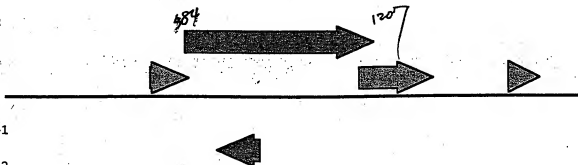
Frame 2

Frame 1

Frame -1

Frame -2

Frame -3



One more ATG @ 484 in frame with ATG starting from 602.

RESEARCH SUMMARY PAGE

1067
McDonnell
GN-000

Gene Name:

All Known Alias Gene Names:

Human: Zhwx00-00001-a1
Rat: Agp-22423-a1

Member of the interferon family of proteins
Name: Interferon-like protein.

Investigator(s):

Initial Date of Summary Preparation:

Duanzhi Wen, Andrew Welcher, Michael
Kelley

Initial invention disclosure filed [REDACTED]
This summary filled in on [REDACTED]

Description of Project:

This novel member of the interferon family of proteins is related to the beta, alpha, and omega subfamilies. As an interferon it would be expected to have anti-infective and anti-proliferative uses. Additionally, it might find use in the treatment of multiple sclerosis and other pathologies requiring immunomodulation.

Gene Nucleotide Sequence:

Human:

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1  CGCGTACGTA AGCTTAATTT AACAAATTTG GAAAAACCTA AACTATACTG
51  TGCTCTGGTG ACCTAGCAAT CAAATAATCA CAGTCATTTG GTCAAATGCTT
101  ATGATTAAGT CAATGAGACA GGATGTTTGG CTATAGCACC AGGTACAAAA
151  AATATATTTT CATGAAGGAT CACTCCCTCT TATGTAATAG ATTTGGGTGA
201  GTGAGTGAGT GAGTGAGTGC ATGGACTCAC AGCTTTTGCC TTCTGGAAT
251  ACCCTGCATC AGTCTTGTTA TGATGATTCC TTAGTGCTGG GATGGATCAT
301  CCAGGCATTT AAGGTAACAC GATGGTAATT CTTTGCTCAT TTTCAGGGA
351  AAAAAAAGAG TTATCACTTC CAAAGTCGGC ATAGTCACCC GAAGTAAAAA
401  AAAAAAAGAG AAAAAAAGC CTCAGAGGCA AAGGAAAGGG GCCGCAACCT
451  TGTTAACTGT TGAATGACG AATGAGAAAA CTCCTCCTGC TGAAGATATT
501  CAGGTATATA AAGGCACATG AAGGAAAACT CAAAACATCA TTGTCATATA
551  CACATCTTCT GGATTTTTTA GCTTGCAAAA AAAATGAGCA CCAAACCTGA
601  TATGATTCAA AAGTGTTTGT GGCTTGAGAT CCTTATGGGT ATATTCAATT
651  CTGGCACCTT ATCCCTGGAC TGTAACCTAC TGAACGTTCA CCTGAGAAGA
701  GTCACTCTGC AAAATCTGAG ACATCTGAGT AGTATGAGCA ATTCATTTC
751  TGTAGAAGTT CTACGAGAAA ACATAGCTTT TGAGTTGCC CAGAGTTTC
801  TGCAATACAC CCAACCTATG AAGAGGGACA TCAAGAAGGC CTTCTATGAA
851  ATGTCCTTAC AGGCCTTCAC CATCTTCAGC CAACACACCT TCAAAATTTG
901  GAAAGAGAGA CACCTCCAAAC AAATCCAAAT AGGACTTGAT CAGCAAGCAG
951  AGTACCTGAA CCAATGCTTG GAGGAAGACG AGAATGAAAA TGAAGACATG
1001  AAAGAAATGA AAGAGAATGA GATGAAACCC TCAGAAGCCA GGGTCCCCCA
1051  GCTGAGCAGC CTGGAAGCTG GAGATATTT CCACAGGATA GACAAATTTCC
1101  TGAAGAGAAA GAAATACAGT GACTGTGCCT GGGAGATTGT CCGAGTGGAA
1151  ATCAGAAGAT GTTTGTATTA CTTTACAAA TTTCAGGAGT TATTGAGGAG
1201  GAAATAAGGT ATATTTTTGG AATTAAATTT CTTTCTCCCT CCGAAATCTC
1251  TTCTCTCTTC TCCTCCTCCA TCTCTTTTT AAGGATTGTT GTGCTCTCTC
1301  GTAAGCCTGT CTTCAAGTTG ACTGGTAGCC TCGGAACATC AGGGACACTC

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1351 ACCTCTCTAA GGAGAGGTAA TGGCAACCAT CCTCAGGGTG ACCAAGAGTC
 1401 TCCTTAGAAA GTCTTTAAGA CATTTTTTAA GGAATAAGAT TCCCTCTCCG
 1451 TCTTCTCTTA TTCTCTCTTG CTCTTTTCTG TGGCCATTTT GAAAGAGCTT
 1501 TGCTATATAT ACCACCTCTG GACTTCACCA AGACAATGGC TAGAGGATAG
 1551 GGAGCAGAGA ATGTGTCAAA ATGGTAAACAT TTCAATGACT TAACGTGTTT
 1601 GCTGCCAAGG TTGCTTATCC TATGAAAATT CAGCACATTA AAAGAGCTTA
 1651 TACATGCTCC CTAGAGTCAA TACTCTTGCA TTTTCCCCCT CCTGCTCGGG
 1701 GGGAAAAAGG TTGACATTTC TGGCCCATTT CTTTCTCAGC GTGGTTTGTT
 1751 TCAATTGATG CTGTGGAAT GGTATTTCAT TACTTTAAGA GTGAAGATCC
 1801 ATAATTGAAT TGGATGGAT GTTGAATTAG ACGACCATTG AGCTTGATTC
 1851 CTCTAGAGCG GCCGCCGACT AGTGAGCTCG TCGACCCGGG AATT

Rat:

1 GGGTGTTGTA GATATTTTTC CTTTGGGAAGA AATACTGAGC ACCAAGGCTG
 51 AGATGACACT GAAGTATTTA TGGCTGGTGG CCCTCGTGGC TCATATACAT
 101 TCACCCATCC AGTCTCAGAA CTGTGTGTAT CTGGATCATA CCATCTTGGA
 151 AAACATGAAA CTCTCTGACA GCATCAGGAC CACCTTTCCC TTAAGATGTC
 201 TAAAGATAT CACGGATTTT GAGTTTCTCT AAGAGATTCT GCTGTACGTC
 251 CAGCATGTGA AAAAGGACAT AAAGGCAGTC ACCTATCATA TATCTTCTCT
 301 GCGGCTAATT ATTTTCAGTC TTAAGAGACT CATCTCCCTG GCGACAGAGG
 351 AACGCTTGGA ACGTATCAGA TCGGGAGCTT TCAAACRAAGT GCAGCAAGCT
 401 CGAGAGTGCA TGTAGACGCA GGAGAACAAG AACACGGAGG AGGACAGTAC
 451 ATCACAACAT CCTCACTCAG AGGGCTTCAA GGCAGTCTAC CTGGAATTGA
 501 ACAAGTATTT CTTCAGAAAT AGAAAGTTCC TGGTAAATAA GAAATACATG
 551 TTCTGTGCGT GGAAGATTGT CGTGTGGAAA ATAAGAAGAT GTTTCAGTAT
 601 ATTTTACAAA CTACTCAACA TGAATTGAGA ATCATCCAGC TTCAAGCAAG
 651 AACTTAGATA GAAAGTTGTA CTGCTCAAA GTCCCCAAGA ACGCTTGATT
 701 CTAAGGCTAT TGCAGTCTG CTGCTACACA CTCCGGACGC AAGACTTTTC
 751 AAGGTCAAGG TTCAAGGTAG TACAGTCAA GGAAGTCTTA TGTTAAGCAA
 801 AAGAAAAATT TCAGTGGAAA AGCTAGCAGA AATGTCAACT TGTCAAAAAA
 851 ACAACTPATG GATTATGGCA TTGACGTTAC TAGCAAAAAA AATAAACAAA
 901 AAAAAACAA AAA

Gene Amino Acid Sequence:

Human:

1 MSTKPDMIQK CLWLEILMGI FIAGTSLDC NLLNVHLRRV TWQNLRLHSS
 51 MSNSFPFVECL RENIAFELPQ EFLQYTQPMK RDIKAFYEM SLQAFNIFSC
 101 HTFKYWKERH LKQIQIGLDQ QAEYLNQCLE EDENENEDMK EMKENEMKFS
 151 EARVPQLSSL ELRRYPFRID NFLKEKKYSYD CAWEIVRVEI RRLYIFYPKF
 201 TALFRK*

Rat:

1 MTLKYLNLVA LVALYSISIQ SQNCVYLDHT ILENMKLLSS IRTTFPLRCL
 51 KDITDFEFPQ EILLYVQHVK KDIAVYTHI SSLALIIFSL KDSISLATSE
 101 RLERINSGLF KQVQQAECM VDEENKNTSE DSTSQHPHSE GPKAVYLELN
 151 KYFFIRIRKFL VNKYSFCAM KIVVVEIRRC FSIFYKLINM N*

Figure Containing cDNA and Amino Acid Sequences:

Human:

Sequence Analysis of Human IFN-novel

[illegible]

A human gene which encodes a novel protein of 207 amino acids was isolated by screening the human genomic DNA library using a rat cDNA clone. The deduced amino acid sequence of this novel gene is indicated below the first nucleotide of each codon, and the termination codon is marked with an asterisk. The protein starts with cysteine, and the signal peptide is underlined. This novel protein is 27% identical to human IFN- β .

Rat:

1	GGGTGTGTAGATATTTTTCCTTGGAGAACTACTGACACCAAGGCTGAGATGACACT	60
1	-	3
		M T L
61	GAAATATTATGGCTGGTGGCCCTCGTGGCTCTATACATTCAACCCATCAGTCTCAGAA	120
4	K Y L W L V A L V R L V I S P T O S O N	23
121	CTGTGTGTATCTGGATCATACCATCTTCGAAACATGAACCTTCGAGCAGCATCAAGAC	180
24	C V Y L D H T I L E N M K L L S S I R T	43
181	CACGTTTCCCTTAAGATGTCTAAAGATATCAGCGATTTCAGTTTCTCTCAGAGATTCT	240
44	T F P L R C L K D I T D F E F F Q E I L	63
241	GCTGTACGTCAGCATGTGAAAAGGACATAAAGCAGTCACCTATCATATATCTTCTCT	300
64	L Y V Q H V K K D I R A V T Y H I S S L	83
301	GGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGGGACAGAGAACGCTTGA	360
84	A L I I F S L K D S I S L A T E E R L E	103
361	ACGTATCAGATCGGACTTTTCAACAAAGTGCAGCAAGCTCAGAGTGCATGATAGACGA	420
104	R I R S G L F K Q V Q Q A R E C M V D E	123
421	GGGAACAGAACACCGAGGAGGACAGTACATCACAACATCTCTACTCAGAGGGCTCAA	480
124	E N K N T E E D S T S Q H P H S E G F K	143
481	GGCGCTCTACCTGGATGAAACAGTATTTCTTCAGAAATCAGAAAGTTCTGGTAAATAA	540
144	A V Y L E L L N K Y F F R I R K F L V N K	163
541	GAAATACAGTTTCTGTGCTGGAGATTGTGCTGGTGGAAATAGAGATGTTTTCAGTAT	600
164	K Y S F C A W K I V V V E I R R C F S I	183
601	ATTTCACAACTACTCAACATGAATTGGAATCATCCAGCTTCAGCAAGCAAGAACTAGATA	660
184	F Y K L L N M N *	192
661	GAAATGTGACTGCTCAAATGTCGCCCAAGACGCTTCGATCTAAGGCTATTGGAGTCTG	720
721	CTGCTACACACTTCGGAGCGACGCTTTCAAGGTCAGGGTTCAGGCAGTACAGTCAAA	780
781	GGAGCTCTATGTTAAGCAAAAGAAAATTTCAGTGGAAAGCTAGCAGAAATGTCACCT	840
841	TGTCAAAAAACAACTTATGGTATTGGCATTGACCTTACTAGCAAAAAATAAACAA	900
901	AAAAAACACAGCTACTAAAAA	923

Cloning Information:

The rat sequence was cloned from a rat placenta cDNA library as part of an EST project and was identified by computer analysis as being a novel member of the interferon family of proteins. Briefly, rat embryo day 17 [E17] placenta mRNA was isolated by standard methods (unnecessary information) (Chomczynski, P. and Sacchi, N., Anal. Biochem. 162, 156, 1987). cDNA was synthesized using the SuperScript Plasmid cDNA kit supplied by GIBCO/BRL and subcloned into the pSPORT1 (GIBCO/BRL) vector into the Sal I and Not I restriction sites.

Cloning of Human IFN-like gene:

Multiple attempts to clone the human IFN-like gene from a variety of human tissue cDNA libraries failed to yield positive clones. However, a human tissue Northern Blot

hybridized with a PCR-generated radioactive rat probe revealed an 1.8 kb Hind III fragment in certain batches of human pancreas mRNA. Attempts to clone this corresponding message in a pancreas cDNA library failed to recover any positive clones.

Examination of the genomic structures of known IFNs revealed that IFN, especially the members in the IFN α family, all share a unique intronless genomic structure. Therefore, screening of human genomic DNA might yield the complete human IFN-like gene. We started with 1×10^6 human lambda genomic clones (Stratagene, Cat. No. 946206) for primary screening at a density of 50,000 clones / plate (*unnecessary information*). Nitrocellulose filters (*unnecessary information*) (S&S) were prepared by standard techniques (Molecular Cloning, A Laboratory Manual, Sambrook, Fritsch, and Maniatis editors).

The following conditions were used.

- Prehybridization and hybridization conditions: 30% formamide, 5x SSC, 2x Denhart's, 10 μ g/ml Salmon sperm DNA, 0.2% SDS, 2mM EDTA and 0.1% pyrophosphate. Hybridization was conducted overnight at 42°C. The washings were done under following conditions: 1x SSC, 0.1% SDS at room temperature for 30-60 minutes followed by 0.2x SSC and 0.1% SDS at 55°C for 15 minutes.
- Generation of radioactive PCR probe (*unnecessary information*): rat cDNA full-length fragment 20ng, primer 1795-01 and 1795-02, 20 pmol each, 1mmol dNTP (dCTP @ 0.01mmol), 32P-dCTP 5 ml and 4mM MgCl₂. Reaction condition: denature at 94°C for 30sec, anneal at 60°C for 30sec and elongate at 72°C for 1 minute. The reaction is repeated for a total of 45 times. Simultaneously a "cold" PCR reaction is performed under exact condition except the dNTP mix is dCTP balanced. The radioactive probe was purified by Quick Spin G-50 column and boiled at 100°C for 10 minutes before chilling on dry ice for 20 minutes. The probe is usually 5×10^5 cpm/ μ l.

Three positive clones were recovered after primary, secondary screening and subsequently purified to homogeneity. The lambda phage DNA was prepared by a solid plate culture method. The NotI insert from these clones were excised out and ligated into pSport (GIBCO BRL) vector and transformed into DH10 E. coli strain. The transformants were prepared by Qiagen Spin Column plasmid prep kit. The plasmid DNA was then digested with HindIII. The digested fragments were resolved on agarose gel and transferred to a nylon membrane for Southern Blot analysis. The analysis was conducted under the same condition genomic screening was carried out. The corresponding fragment recognized by "hot" rat probe was then subcloned in pSport vector for sequencing analysis. According to the HindIII digestion pattern, we determined these three independent clones were likely to contain identical genomic insert. The sequencing analysis confirmed our speculation. This 1.8kb HindIII fragment contains an open reading frame of 624 base pairs that has 64% similarity to the sequence of rat mrpe3-00078-F6-Wz. In terms of similarity in amino acid sequence, the human sequence is 40.5% identical to and 50% similar to that of rat. All 5 predicted Cysteine residues were perfectly aligned with those in rat protein sequence. Moreover, the human sequence is predicted to contain a signal peptide and cleavage site. The human IFN-like protein is strongly predicted to resemble a secreted cytokine molecule (91% probability).

Amino Acid Sequence Alignment of Human IFN-novel, Rat IFN-novel and Human IFN- β

- Human IFN- η is most close to human IFN- β , with 30% identity. Four out of five cysteine residues are conserved between them.

Northern blot analysis detected IFN-like mRNA in several different stages of mouse and rat embryos. Northern blots used RNA isolated as above. The full-length rat cDNA was used as a probe. Prehyb conditions were 40 % formamide, 5X SSC, 1 mM EDTA, 0.1 % SDS, for 4 h at 42°C. Hyb conditions were the same as above except were done overnight at 42°C. Blots were washed with 0.2x SSC, 1 mM EDTA, and 0.1% SDS for 30 min at 60°C.

RT-PCR (conditions are not necessary - standard technology) identified IFN-like mRNA in the following human tissues : pancreas, small intestine, prostate, uterus, thyroid, and placenta.

Production of human and rat IFN-like protein in *E. coli* :

Waiting on data from Karen Sitney. However, the E. coli protein did not appear to be folded correctly and has not yet generated any biologically active material.

Production of human and rat IFN-like protein in a mammalian expression system:

Several versions of the human and rat IFN-like protein have been produced in a mammalian expression system (either CHO or 293 cells). The proteins synthesized were either the native protein itself, or a native protein-Fc fusion. Some of the Fc fusion constructs contained a cleavage site which allows the native protein to be released from the Fc portion after being produced in the conditioned media of CHO cells.

PCR amplification of IFN-like molecule:

PCR primers were selected to amplify the coding sequence of rat/human IFN-like molecule:

Rat IFN-Like Molecule primers:

- IFN-Like molecule Fc-fusion:

1847-77 CCC AAG CTT ACC ATG ACA CTG AAG TAT TTA TG

Forward primer: Hind III site plus ATG

1847-78 AAG GAA AAA AGC GGC CGC ATT CAT GTT GAG TAG

- Reverse primer: Not I site and no stop codon for Fc fusion

Soluble IFN-like molecule:

1896-56 ACG CGT CGA CTC ATC AAT TCA TGT TGA GTA GTT TG

Reverse primer: Sal I site plus 2 stop codons (for pDSR α cloning).

1896-57 AAG GAA AAA AGC GGC CGC TCA TCA ATT CAT GTT GAG TAG

Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

Human IFN-like primers:

- Soluble human IFN-like primers:

1954-45 ACG CGT CGA CTT ATT ATT TCC TCC TGA ATA G

Reverse primer: Sal I site plus 2 stop codons (for pDSR α cloning).

1954-46 AAG GAA AAA AGC GGC CGC TTA TTA TTT CCT CCT GAA TAG AGC

Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

- Human IFN like-Fc fusion primers:

1955-44 CCC AAG CTT ACC ATG AGC ACC AAA CCT GAT ATG

Forward primer: Hind III site with 1st ATG

1954-47 CCC AAG CTT ACC ATG ATT CAA AAG TGT TTG TGG C

Forward primer: Hind III site with 2nd ATG

1954-48 AAG GAA AAA AGC GGC CGC GCG GCC CTC GAT TTT CCT CCT GAA TAG AGC TGT AA

Reverse primer: Not I site, no stop codon with *Factor Xa* cleavage site and Fc fusion

1954-49 AAG GAA AAA AGC GGC CGC TTT CCT CCT GAA TAG AGC TGT AA

Reverse primer: Not I site and no stop codon for Fc fusion

PCR Reaction:

Rat:

Reaction Mixture: template 20 ng, 1847-77 and 1847-88 or 1896-56/57, 20 pmol each, 1mmol dNTPs, 4mM MgCl₂, 1X PCR buffer, 5u Taq polymerase.

Reaction condition: 2 cycle-linked PCR.

94 °C for 30 sec, 50 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94

°C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

Human interferon-like protein PCR conditions:

Reaction Mixture: template 20 ng, 1955-44 and 1954-45 or 1954-46 (soluble form) or 1945-48/49 (Fc fusion), 20 pmol each, 1mmol dNTPs, 4mM MgCl₂, 1X PCR buffer, 5u Taq polymerase.

Reaction condition: 2 cycle-linked PCR.

94 °C for 30 °C sec, 48 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94 °C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

While 1955-44 primer generates an ORF using first Met in the coding region, a separate PCR with 1954-47 to obtain an insert using 2nd downstream Met was also generated. But in terms of secretion efficiency, when tested in 293 EBNA transient transfection, there was no detectable difference could be defined.

For both rat and human, the PCR products were purified by Qiagen PCR purification spin column and subjected to restriction digestion by respective enzymes (HindIII and NotI (pCEP4) or SalI(pDSRα)). After digestion, the fragment was purified from agarose gel with Qiagen gel purification spin column. The purified fragment was quantified and ligated into pCEP4 (for native form), pCEP4-Fc (for Fc form) or pDSRα (native form or Fc form) vectors respectively. The ligation was transformed into DH10. The transformants were picked for miniprep and subsequent sequencing verification. Accuracy of each cloning fragment was verified by sequencing including the Fc junction sequence. The clone was then maxi-prepared for tissue culture transfection experiments. The IFN-Fc fragment in pCEP4-Fc vector can be released by cutting this vector with HindIII and SalI and re-ligated this fragment into pre-digested pDSRα to yield a vector suitable to transfect CHOD⁻ cells.

Transfection:

- Protocol for transfection into 293 EBNA and CHO cells with lipofectin was adopted from the one used by Jin Cao. Same protocol was used to generate both transient and stable transfectants.
- A commercial available calcium phosphate transfection kit was used in CHO cell stable transfection (protocol is attached).
- A CHO cell transfection and selection protocol from Yi Luo was utilized, except calcium phosphate transfection procedure, which has a commercially available kit.

In general, lipofectin transfection yields more stable transfection colonies. Those colonies express comparable level of secreted proteins as those picked from calcium phosphate method.

Generate conditioned media containing recombinant protein.

In order to conduct functional studies on this interferon-like molecule, large quantity of conditioned media (CM) were generated from a pool of hygromycin selected 293 EBNA clones. The cells were cultured in Nunc Triple Flask (500cm) to 80% confluence before switching to serum free media for a week before harvesting. The CM was then sent to purification with protein A affinity chromatography. The purified protein was then used to generate a rabbit polyclonal antibody and to test for in vitro activities. The processing of signal peptide as well as partial amino acid sequence was verified by peptide sequencing.

Purification of human IFN-like-Fc

Conditioned media from CHO cells expressing huIFLM-Fc was thawed and 0.2µm filtered. The filtered material was loaded onto a Protein G column that was previously equilibrated with PBS, pH 7.0. After loading, the column was washed with PBS until the absorbance at A₂₂₀ reached baseline. The protein was eluted from the column with 0.1M Glycine-HCl pH 2.7 and immediately neutralized with 1M Tris-HCl pH 8.5. Fractions containing huIFLM-Fc were pooled and dialyzed into PBS and stored at -70°C.

Factor Xa cleavage of human IFN-like-Fc

The human IFN-like-Fc construct has a Factor Xa cleavage site (IEGR) inserted between the Fc and huIFLM. This site is cleaved with restriction protease factor Xa. The human IFN-like-Fc in PBS was dialyzed into 50mM Tris-HCl, 100mM NaCl, 2mM CaCl₂, pH 8.0. The Factor Xa was added to the dialyzed protein at 1/100 (w/w). The sample was incubated overnight at room temperature.

Abs (available, ordered, proposed):

1. Polyclonal:

Polyclonal antibodies were prepared using both rat and human proteins produced in E. coli and CHO cells (from above) using standard immunological techniques. Antisera were positive for the proteins as determined by Western blot analysis (standard techniques)

2.

Monoclonal:

None.

3. Peptides:

None.

Phenotype and/or Biological Activity:

1. Transgenic /

(pending / analyzed)

other	
<p>Because the lack of a phenotype constitutes a 'negative' result no conclusions can be drawn from this experiment. Further testing will be required to determine any or all of IFN-like proteins' biological activities in vivo.</p>	
2. <i>in vivo</i> assays:	(available, used, proposed)
<p>Not done.</p>	
3. <i>in vitro</i> assays:	(available, used, proposed)
<p>Rat IFN-like Fc fusion protein treatment of several cell lines caused phosphorylation of some cellular proteins (unidentified).</p>	

References:

Nothing specifically published on this gene. Lots of references for the interferon family.

Genomic DNA Sequence (i.e. including all introns and exons):

The human gene was cloned from genomic DNA. The attached sequence (above) comes from genomic DNA and includes the coding region which is found in one exon, and the flanking regions.

Ortholog DNA Sequences:

Human and rat sequences cloned.